# GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND
VARIATION

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## CORRIGENDA

### VOLUME 12, 1927

Page 503, table 3, heading, for "$Y_gW_z$" read "$Y_g-W_z$.

Page 504, chromosome map, for "$g^v$" read "$y_g$."

Page 511, table 10, under progeny number, last figure, for "4993" read "4493."

Page 530, Table of contents, line 9 and page 546 subtitle, for Imperfect dominance of "*f*," read Imperfect dominance of "*F*."

Page 536, line 13, for "pink-browns" read "pink-eyed browns." Line 15, for "*PPbb*" read "*Ppbb*."

Page 549, table 14, under column "change in grade," 8th entry, for "0.0" read "1.0."

Page 550, table 15, under column "change in grade," first entry, for "2.3" read "3.3."

Page 556, table 17, second line in heading, for "$.6745\ \sigma/n$" read "$.6745\ \sigma/\sqrt{n}$."

Page 566, last line, enclose expression "$c^a \cdots Fpb$" in parenthesis.

### VOLUME 13, 1928

Page 2, line 21, after Navaschin for "1926" read "1925."

Page 11, line 4, of legend to figure 2, for "ate" read "late".

Page 41, line 13, after "type" put semicolon instead of comma.

Page 43, line 25 after Winkler, H., for "1920" read "1910."

Page 86 and 87, formulas 4 and 5 respectively, for "$d$" read "$d/n$."

Page 91, table 4 coupling phase, under Yule's $Q$,

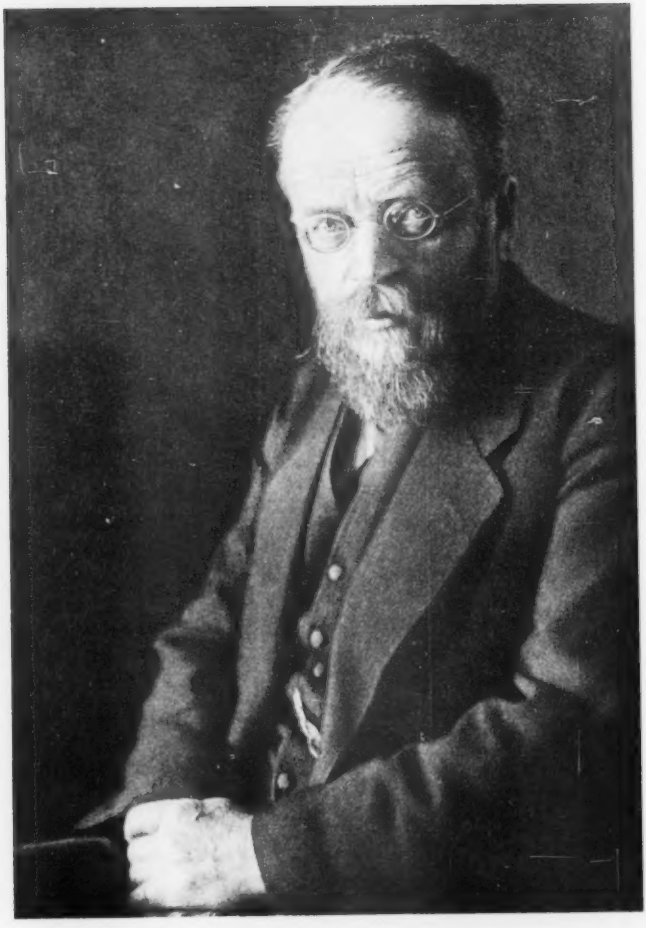
| for | read |
|---|---|
| 74.32 | 75.68 |
| 73.59 | 76.41 |
| 74.98 | 75.02 |
| 77.42 | 72.58 |

Page 95, line 22, for "7.6" read "5.7."

Page 95, line 18, for "$x^2=5.27$" read "$x^2=5.72$."

Page 189, Literature cited, for "Metz, C. A." read "Metz, C. W."

Page 419, first line of summary, for "test" read "tests."



Mit freundlichem Empfehlungen
Ihr ergebener
C. Correns.

# CARL CORRENS

(FRONTISPIECE)

GEHEIMRAT PROFESSOR DOCTOR CARL (ERICH) CORRENS was born in München, Germany, September 19, 1864, the only son of a distinguished painter, ERICH CORRENS and his wife EMILIE CORRENS née Köchlin, also a gifted painter. Orphaned by the early death of both parents,—his father in 1877 and his mother in 1881,—CORRENS was placed in an orphanage at Augsburg in Bavaria, but after spending six months in the gymnasium there his health broke and he was sent to a tuberculosis sanitarium and only a year later resumed his studies in ST. GALLEN, Switzerland, where he was in the care of FRÄULEIN KÖCHLIN, his mother's sister, who saw him successfully through his school years. He graduated from ST. GALLEN with high standing in 1885.

When he entered the UNIVERSITY OF MÜNCHEN he had already a knowledge of general botany and a thorough floristic knowledge of the flowering plants and mosses; but he also had a strong inclination toward chemistry.

At the UNIVERSITY, CARL VON NÄGELI had already ceased to lecture so that CORREN's courses were taken with the Docents, A. PETER, H. DINGLER O. LOEW; but NÄGELI had known CORREN's parents and took an interest in him, advised him in his studies and particularly incited him to study the Algae. The last summer semester of his preparation for the Doctorate was spent at GRAZ with DR. G. HABERLANDT, who took great interest in him. In March 1889 he won his Doctor's degree with a thesis" Ueber Dickenwachstum durch Intussusception bei einiger Algenmembranen," the subject for which had been suggested by NÄGELI. His minor subjects were Physics and Chemistry. After receiving his degree he spent another semester at GRAZ with HABERLANDT, studying the anatomical adaptations of flowers toward visiting insects. Two semesters he was in Berlin in SCHWENDENER's INSTITUT, continuing his studies on the growth of cell membranes; and then for a brief period with PFEFFER in LEIPZIG studying the phenomena of irritability.

In the spring of 1892 he took a position as Privatdozent at the UNIVERSITY OF TÜBINGEN with VÖCHTING, and the same year he was married to ELISABETH WIDMER, a grandniece of NÄGELI. Throughout his scientific career his wife has been his capable and active research assistant as well as the mother of his three children. Under the direction of NÄGELI she had prepared and published (1891) before their marriage a monograph on the European species of Primula.

During his ten years as Privatdozent at TUBINGEN, CORREN's work was continued chiefly along the physiological lines he had already started, but in addition he began to make use of the botanical garden for experimental cultures. In the middle nineties he began his studies on Xenia, using maize, stocks, beans, peas and lilies. The sharp segregations displayed by the maize kernels on the same cob, and of pea seeds within the same pod, impelled the making of statistical studies which led to the discovery of MENDEL's laws. With four filial generations of peas before him, he arrived by a sudden inspiration at the correct interpretation, and then made the search through the literature which led to the discovery, with the help of FOCKE's Pflanzen-Mischlinge, that MENDEL had arrived at the same conclusions thirty-five years before.

In the spring of 1902 CORRENS accepted a call to LEIPZIG as Professor extraordinarius. In 1909 he went to MUNSTER I. W. as Professor ordinarius

and in 1913 was appointed first Director of the KAISER WILHELM INSTITUT FÜR BIOLOGIE in Berlin-Dahlem, where he is still active, having established one of the most important centers of genetical research in the world.

The publication of his classic work on Xenia which opened his genetical career in 1899; his independent rediscovery of Mendel's laws, announced in 1900 simultaneously with similar announcements of DE VRIES and TSCHERMAK; and a 200-page paper on maize hybrids published in 1901 placed him at once in a position of leadership which he has maintained consistently ever since. He has not hesitated to attack the most difficult genetical problems, involving sex, variegation, self-sterility, heterostyly, differential pollen-tube growth, experimental modification of sex-ratios, etc.

To celebrate his sixtieth birthday the DEUTSCHEN GESELSCHAFT FÜR VEREBUNGSWISSENSCHAFT collected and published in 1924 in a single volume of about 1300 pages all his genetical papers, sixty in number, except two independent publications on sex.

CORRENS was awarded in 1912 the SÖMMERING medal of the SENCKENBERGISCHEN NATURFORSCHENDEN GESELLSCHAFT, and the same year received the civil Order of the Red Eagle, 4th Class, and in 1915 the title "Geheimer Regierungsrat." Honorary doctorates have been conferred on him in Medicine (ROSTOCK), Agriculture (LANDWIRTSCHAFTLICHE HOCHSCHULE BERLIN," Civics (MÜNSTER I. WESTFALEN), and natural science(TÜBINGEN). He is a Member of the PRUSSIAN ACADEMY OF SCIENCE, Non-resident or Corresponding Member of the SCIENTIFIC SOCIETY IN GÖTTINGEN, the ACADEMIES in Vienna, München and Stockholm, and Corresponding Member or Honorary Member of the DEUTSCHEN GESELLSCHAFT FUR VERERBUNGSWISSENSCHAFT, the BOTANICAL SOCIETY OF AMERICA, the BOTANICAL SOCIETY OF TOKYO, etc., etc.

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# A CYTOLOGICAL AND GENETICAL STUDY OF PROGENIES OF TRIPLOID TOMATOES[1]

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## INTRODUCTION

Perhaps the most familiar kind of mutation is that which appears to involve a change at a specific locus in a chromosome, commonly called a gene mutation. No change of a quantitative kind is visible in the chromosomes. Mutant characters of this kind were used by MENDEL in his famous "Experiments in plant hybridization." As a general rule, such mutants can be made to breed true. Another kind of mutation is that associated with a change in the number of chromosomes. Usually one or more complete sets of extra chromosomes are present as in polyploids or a smaller number is present in addition to the normal complement. As a rule, except in the case of certain types of polyploidy, these chromosomal mutants are genetically inconstant and cannot be made to breed true.

[1] Paper No. 164, UNIVERSITY OF CALIFORNIA, Citrus Experiment Station, Riverside, California.

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Doubtless chromosomal mutants were found by the hybridists and earlier geneticists and, before the advent of the chromosome theory, these must have been a source of much perplexity both from their elusive character and from their peculiar mode of inheritance. Probably in many cases they were thought to be the result of accident or disease. Recent work seems to have brought to light an essential similarity between chromosomal and gene mutations. According to the theory of gene balance which we owe chiefly to BRIDGES (1921), the characteristics of mutants of both kinds are an expression of a certain gene balance. While a gene mutant appears to result from a qualitative change at a certain locus in the chromosome and a chromosomal mutant from a quantitative change occasioned by the change in chromosome number, in both the genes play an essential part in determining the mutant characters.

While a diploid plant contains $2n$ chromosomes, where $n$ represents the number of pairs present in the heterotype division up to the beginning of the anaphase, a triploid contains $3n$ chromosomes, or $n$ sets each consisting of 3 chromosomes which tend to attract one another. According to a recent list (LESLEY, MARGARET M., 1926), triploidy has been reported in Oenothera, Morus, Uvularia, Datura, Canna, hyacinth and tomato; to this, maize (RANDOLPH and MCCLINTOCK 1926), Primula (IINUMA 1926) and Crepis (NAVASCHIN 1926) may now be added. Apparent triploid plants originating from species crosses followed by parthenogenesis as in the polyploid roses studied by TÄCKHOLM (1922) are not necessarily triploids in the strict sense.

A triploid plant is usually a replica of the corresponding diploid but is distinguished by the larger size of the organs, cells and nuclei. Usually the pollen is scanty and contains many empty grains. Little viable seed is produced.

BLAKESLEE (1924) has shown that the progeny of a triploid Datura includes a remarkable series of forms with one or more extra chromosome. Although different in origin, these forms are undoubtedly of the same nature as certain of the mutants which regularly appear among the progeny of normal Datura plants.

A triploid tomato plant which chanced to appear in a commercial variety was the starting point of the present work. As in the case of Datura, crosses between triploid and diploid tomato plants gave rise to a series of variant types comparable with chromosomal mutants. The number of chromosomes has been determined and the characteristics and genetic behavior of certain of these types have been studied with the special object of throwing some light on the composition of the individual chromosomes.

ACKNOWLEDGMENTS

The writer is deeply indebted to his wife, Doctor MARGARET MANN LESLEY, for much help especially in the cytological work. He has also received valuable suggestions from Professor ROY E. CLAUSEN, Division of Genetics, UNIVERSITY OF CALIFORNIA. For advice in the preparation of this manuscript the writer is indebted to Professor E. B. BABCOCK, Division of Genetics, UNIVERSITY OF CALIFORNIA, and to Doctor HOWARD B. FROST, Citrus Experiment Station, UNIVERSITY OF CALIFORNIA.

## METHODS

Chromosome number was, as a rule, obtained from smears of pollen mother cells, stained by Belling's method. The most generally favorable stage for counting was the second metaphase but cells in which both plates are countable are sometimes difficult to find; diakinesis was often used and occasionally interkinesis and second anaphase. First metaphase and anaphase often gave useful indications. In exceptional cases to be noted later, chromosomal counts were obtained from sections of root tips and of anthers fixed in chrom-acetic-urea and stained in Heidenhain's iron-alum haematoxylin. In no case was a constant discrepancy found between chromosome number in the root tips and in the pollen mother cells, but the occasional presence of tetraploid pollen mother cells in diploid or trisomic plants previously reported by M. M. LESLEY (1925) was confirmed. Although the chromosomes are only about $1\mu$ in diameter at the second metaphase, their relatively small number and their shortness in the later phases of meiosis and in root tips, make the tomato fairly favorable material for chromosome counting.

In many first-metaphase plates there is some gradation in chromosome size; in particular one large bivalent is often seen at the periphery and smaller ones in the center of the plate. These differences, however, are not constant and are probably due to slight differences in stage of development; they were not detected in root tips.

The pollen was examined in acetocarmine. Mixed samples were taken from what appeared to be typical healthy and ripe anthers. The grains which were round and full of stained material were counted as "good" and those which were irregular in outline, imperfectly filled or empty were counted as "bad." Very few burst grains were found. In the same plant and even in individual anthers of the same flower the variability both in quantity of pollen and in proportion of good grains greatly exceeded that of random sampling.

Unless otherwise stated, all flowers in both self- and cross-pollination were inclosed in paper bags and the usual precautions taken in pollination. Field cultures were for the most part grown in the experimental grounds of the Division of Genetics, at Berkeley, and greenhouse cultures at the CITRUS EXPERIMENT STATION, Riverside.

## ORIGIN OF TRIPLOIDS AND $F_1$ PROGENY

The normal tomato plant was found by WINKLER (1910) to contain 24 chromosomes in the root tips and 12 pairs at diakinesis in the pollen mother cells. In 1923 a single triploid plant was found among 29 plants of Livingston's Dwarf Aristocrat, grown from commercial seed. The root tips contained 36 chromosomes and a study of the prophases of meiosis in the pollen mother cells by M. M. LESLEY (1926) showed at diakinesis, as a rule, 12 trivalents. The morphological characteristics of this plant have been briefly described in a previous paper (LESLEY and MANN 1925). The stems, leaves and flowers were more or less gigantic but the fruits were undersized and few in number. In 1924 a second triploid plant identical in appearance with the first occurred among 85 plants from the same lot of seed (figure 3). A third appeared among 104 plants also from commercial seed of a rather similar variety, Dwarf Champion, and a fourth in a commercial planting of an early strain of Stone, a variety of standard habit. Altogether among 200 plants of Dwarf Aristocrat 2 plants (1 percent) and among 280 plants of Dwarf Champion 1 plant (0.4 percent) were triploid. Some of the giant unfruitful plants known to commercial tomato growers as "he" plants or "fruitless hybrids" are undoubtedly triploids, others perhaps are trisomics as is noted below (p. 36).

A triploid plant may originate from a cross between a tetraploid and a diploid. Tetraploid tomato plants were obtained by WINKLER (1916) from shoots arising from the callus of a graft between *Solanum nigrum* and tomato. He reports regular 24+24 reduction in the pollen mother cells but only 5 percent of good pollen. No tetraploid plant has been found in the course of the present study. Recently M. M. LESLEY (1925) has found islands of tetraploid cells in roots and groups of tetraploid pollen mother cells which are believed to have originated from tetraploid islands in the meristems of shoots. She also found diploid pollen mother cells of tomato in which the reduction division was suppressed with resulting dyad formation (unpublished data). In each case a diploid gamete would be formed, which, when fertilized by a normal (haploid) gamete, would give a triploid plant. Probably triploids have arisen in both ways.

The two triploid plants of Dwarf Aristocrat used in practically all the breeding work showed the dwarf habit, rather dark green foliage and smooth scarlet fruit characteristic of this variety. Numerous attempts were made to obtain seed from self-fertilization but without success. Seeds from a few fruits which set on one plant late in the season without hand-pollination gave rise to plants of standard habit and therefore resulted from vicinism. Seed was produced very sparingly from diploid ♀ × triploid ♂, and therefore the failure to obtain seed from self-fertilization seems to be due to defective pollen. Indeed very little mature pollen is found in the anthers of the triploid and a very large proportion of the grains are empty or clearly degenerate (table 5). Triploid Datura produced very little good pollen but gave some progeny from self-fertilization. Seed was readily obtained from triploid tomato ♀ × diploid ♂. In 56 fruits obtained from crosses of this type the mean number of viable seeds per fruit was about 4, as compared with about 50 in diploid Dwarf Aristocrat selfed.

Since pollination with a diploid proved to be the most effective method of obtaining progeny a choice of diploid pollen parents had to be made. Clearly the identification of forms with extra chromosomes was of primary importance. How greatly identification of a trisomic form may be hindered by differences resulting from the segregation of genes has been clearly pointed out by CLAUSEN and GOODSPEED (1924). In the present case, therefore, since self-fertilization had failed, the triploids were crossed with a diploid plant *of the same variety*.

The progeny of triploid × diploid Dwarf Aristocrat will be referred to as the non-hybrid $F_1$. This term is not strictly correct since the triploid and diploid plants were probably not genotypically identical. The two diploid plants used appeared to be typical of the variety, were similar in appearance and probably belonged to the same pure line, but both may have differed genotypically from the triploids. Among the 10 plants with the diploid chromosome number which appeared in the non-hybrid $F_1$, the only obvious variation was in foliage color. In the non-hybrid progenies there was rarely any difficulty in identifying healthy diploid plants in the field or greenhouse. It appears, therefore, that the genotypic differences in the non-hybrid progeny were not sufficient to obscure the differences associated with the presence of extra chromosomes. Small hybrid $F_1$ progenies were also grown from triploid crossed with the varieties Globe, Red Pear, and Magnus, for comparison of the hybrid with the corresponding non-hybrid forms.

The $F_1$ triploid×diploid progenies contained a most striking diversity of types, varying in plant size, color and shape of foliage, flower structure, fruit shape and other characters. Part of these plants were grown in the field and part in the greenhouse. Certain characteristics, especially of foliage, were more evident in the field than in the greenhouse, so that the identification of types, particularly by foliage, was sometimes difficult (see figures 7 and 8). BLAKESLEE and FARNHAM (1923) report a similar experience with the Poinsettia mutant of Datura.

### CHROMOSOME NUMBER OF $F_1$ PROGENY OF TRIPLOID×DIPLOID

Table 1 shows the chromosome number of the hybrid and non-hybrid progenies of triploid ♀ × diploid ♂. M. M. LESLEY (1926) has shown that in the pollen mother cells of triploid tomato the unpaired chromosomes are distributed at random at the heterotypic division, and consequently it might be expected that the chromosome number in the microspores would show approximately a binomial distribution. Such a distribution was also found by BELLING and BLAKESLEE (1922) in the pollen mother cells of Datura and by BELLING (1924) in hyacinth. It is at least not improbable

TABLE 1

*Chromosome number of $F_1$ progeny of triploid×diploid tomato.*

| Number of chromosomes | 2n | 2n+1 | 2n+1 or 2n+2 | 2n+2 | 2n+3 |
|---|---|---|---|---|---|
| Frequencies observed | 7 | 26[2] | – | 7 | 2 |
| | 3[3] | 9[3] | 5[3] | 7[3] | |
| Total frequencies | 10 | 35 | 5 | 14 | 2 |

that the chromosome number of the eggs in these triploids has a similar distribution. It is evident from table 1 that, in tomato, the chromosome number of the progeny has quite a different distribution. In a binomial series, 30-chromosome plants would be the most frequent, but no 30-chromosome plant appeared and no gamete with more than 3 extra chromosomes has given a viable zygote. Some of the counts are not decisive but the data certainly warrant the conclusion that the 25-chromosome plant or simple trisomic is the most frequent and suggest that the

[2] Including one plant with additional fragment.

[3] Based on fewer counts.

double trisomic, diploid and triple trisomic are next in order. Since chromosome counts were obtained from only about half the population, these data represent only a sample of the $F_1$ progeny grown. Probably the frequency of 26- and 27-chromosome plants is underestimated since, as a rule, they produce few or even no buds and relatively few counts were made from root tips. On the other hand a few plants which were obviously diploid were not examined cytologically. One plant was found to contain a whole extra chromosome and an additional fragment.[4] This and other fragment-containing types which occurred in the progeny of double trisomics will be described in another paper. No plant was found to have less than 24 chromosomes.

Presumably the numerous, more or less undersized and abortive seeds which are to be found in the fruit of triploids, represent the inviable combinations with high chromosome number, if indeed the eggs with high chromosome numbers ever became fertilized.

A correlation was found between seed size and chromosome number similar to that observed by FROST (1919) in Matthiola. Non-hybrid $F_1$ seeds were arbitrarily grouped into "large" and "small." The chromosome numbers of the resulting plants are shown in table 2. The data indicate that "small" seeds give rise to a larger proportion of 26- and 27-chromosome plants. One "large" seed however gave rise to a 26-chromosome plant that was extremely abnormal in development.

TABLE 2

*Seed size and chromosome number of $F_1$ plants from triploid×diploid tomato.*

| SIZE OF SEED | NUMBER OF SEEDS | DIPLOID (PERCENT) | TRISOMIC (PERCENT) | ONE EXTRA CHROMOSOME[5] (PERCENT) | MORE THAN ONE EXTRA CHROMOSOME[5] (PERCENT) |
|---|---|---|---|---|---|
| "Large" | 28 | 21 | 79 | 57 | 14 |
| "Small" | 22 | 5 | 95 | 50 | 32 |

As a result of numerous attempts, only 2 plants have been obtained from diploid pollinated by triploid. These originated from diploid×triploid Dwarf Champion and both $F_1$ plants were diploid.

[4] The number of chromosomes in this plant, and in the 27-chromosome and some 26-chromosome plants was determined from root tips by Doctor M. M. LESLEY.

[5] Excluding plants with extra chromosomes of which the number was not positively determined.

*Discussion of chromosome number*

WINKLER (1916) first suggested that hyperdiploid forms were likely to differ more than tetraploids from the normal on account of unbalance in chromosome number.

In the progeny of Datura triploid×self and triploid×diploid, BLAKESLEE and BELLING (1924) found no plant with more than 3 extra chromosomes, although the distribution of the chromosomes in meiosis in the pollen mother cells was approximately binomial. As all combinations with more than 27 chromosomes were absent, it was concluded that an unbalance exceeding 3/24 was inconsistent with viability. In the progenies of the triploid *Oenothera semigigas*×*O. Lamarckiana* and *O. biennis*, VAN OVEREEM (1920) found plants with chromosome number ranging in unbroken series from 14 (diploid) to 20. In the progeny of *O. semigigas*×mut. *velutina*, DE VRIES and BOEDIJN (1924) found a similar range of chromosome number with a mode in the 15-chromosome class. In this species, unbalance in chromosome number appears to have no obvious relation to viability; this seems to point to a certain similarity of its chromosomes at least in genes affecting viability. Presumably the greatest unbalance in chromosome number in these plants is 3/14 and 4/14, for, if 7 is the basal number, a 20-chromosome plant would be less unbalanced than one with 17 or 18 chromosomes.

A glance at table 1 at once suggests that in tomato, as in Datura, viability has some relation to unbalance of chromosome number and is at least an approximate measure of it. It appears that an unbalance of 3/24 is the maximum consistent with viability and that, as a rule, the greater the unbalance, the less the chance of viability of gamete or zygote. It is probable that 27-chromosome plants occur more frequently in the $F_1$ from triploid×diploid in tomato than in Datura, since 2 such plants were found among 66 $F_1$ plants in tomato while BLAKESLEE and BELLING (1924) mention only 1 among a much larger $F_1$ progeny in Datura. This suggests that Datura is more sensitive to changes of numerical chromosome balance than tomato.

BRIDGES (1923) found in Drosophila that triplo-X or triplo-IV or haplo-IV flies were viable but that no triplo-II or triplo-III appeared in the progeny of triploids. Since the numerical chromosome unbalance is the same in triplo-IV and triplo-III, it appeared that the size of the extra chromosome had to be taken into account. According to BRIDGES (1922) the effect of extra chromosomes on development depends on their internal gene balance. It is inferred that the internal gene balance differs in the

chromosomes of the same and of different species. Except in the cases of polyploidy and of replication of the same chromosome, viability will depend, not on the unbalance in terms of chromosome number, but rather on the gene unbalance of the individual chromosomes and combinations of chromosomes. This hypothesis explains why a gamete of Oenothera can sustain an unbalance of 3/7 but a gamete of tomato probably only an unbalance of 3/12.

## BEHAVIOR OF EXTRA CHROMOSOMES

The mode of origin of the triploid×diploid $F_1$ and the presence of trisomes in meiosis of 25- and 26-chromosome plants suggest that the tomato plants with extra chromosomes are, in BLAKESLEE'S (1921a) terminology, simple, double and triple trisomics. Although double trisomics were less extensively studied than simple trisomics the evidence indicates no great difference in the behavior of the extra chromosomes in these two groups. Neither has any constant difference in the behavior of the extra chromosome been observed in different simple-trisomic types.

In diakinesis the arrangement of the set of 3 chromosomes in the pollen mother cells of a simple trisomic is the same as that found by M. M. LESLEY (1926) in her study of the prophases of the triploid tomato. Either 11 pairs and 1 trivalent, the latter arranged in a single row or in a triangle (figure 2A), are present, or less frequently 12 pairs and 1 unpaired chromosome (figure 1A). Usually one member of the set of 3 chromosomes lags during the formation of the first metaphase plate, as commonly one monovalent is seen outside the plate in lateral view of the first metaphase. Sometimes however, the association typical of diakinesis is maintained and 11 pairs and 1 fairly compact trivalent appear; the latter has sometimes been seen as a compact row of 3 in lateral view. In polar views, accordingly, 12 bivalents and 1 monovalent (figure 2B) or 11 bivalents and 1 trivalent (figure 1C) were found. One or two laggards are common in the early first anaphase (figure 1D). It is probable that the unpaired chromosome usually passes without division into one of the second-metaphase plates, as 12+13 (figure 2C) is more common that 13+13 at that stage. A lagging chromosome is usually included in one of the second-metaphase plates, as it is exceptional to find 12+12 at that stage. Sometimes, however, during interkinesis one or two (figure 2D) micronuclei or karyosomes are seen with membranes and chromatin in a condition similar to interkinesis. In such a case the lagging chromatin doubtless fails to reach the second-metaphase plates and forms microcytes

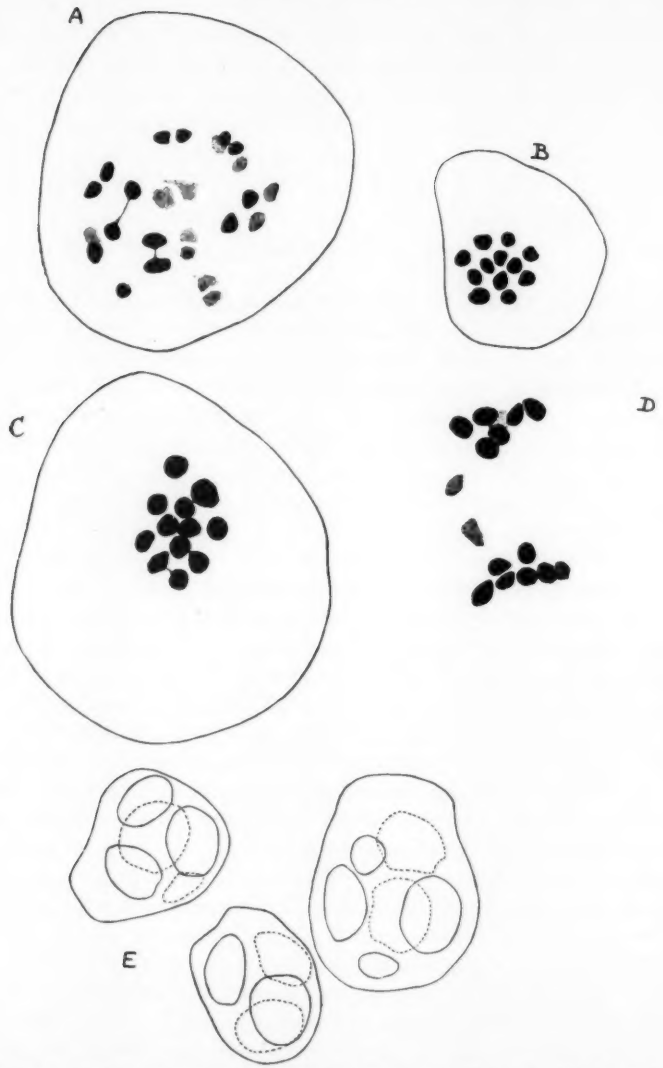


FIGURE 1.—Pollen maturation in diploid and simple trisomics. A, triplo-A, diakinesis, 12 bivalents (pairs) and 1 univalent; B, diploid, 1st metaphase, 12 bivalents; C, triplo-A, 1st metaphase, 11 bivalents and 1 trivalent; D, triplo-E, late 1st anaphase, 2 laggards from division of unpaired chromosome (polar groups not countable); E, triplo-A, tetrad (below), pentad (left), hexad (right). A, C, D×4000; B, E×2200.

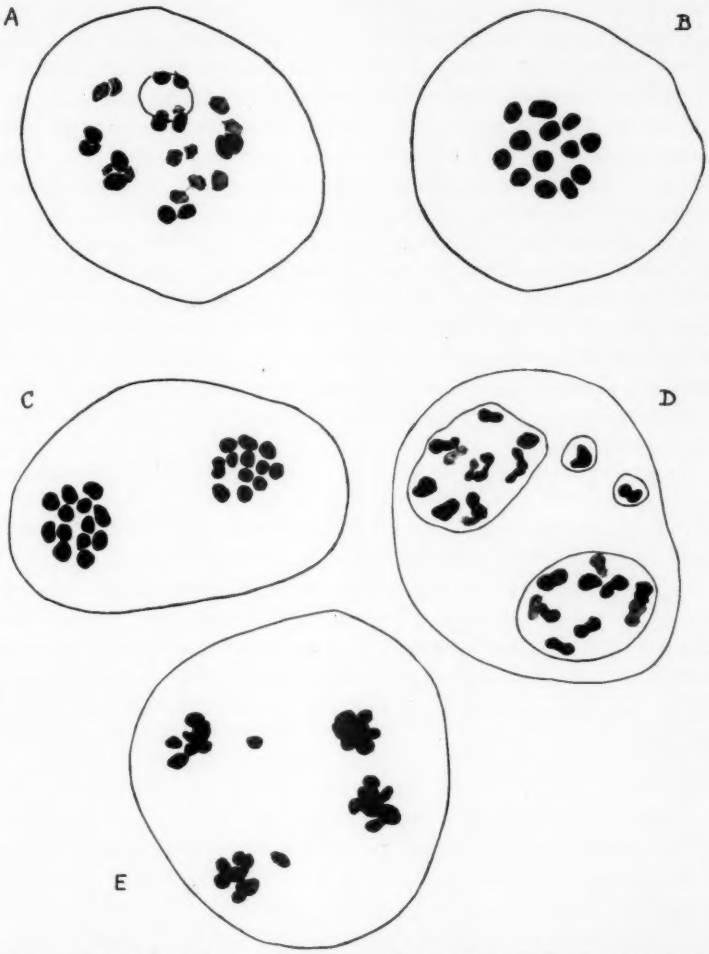


FIGURE 2.—Pollen maturation in simple trisomics. A, triplo-C, diakinesis, 11 bivalents and 1 trivalent; B, triplo-A, 1st metaphse, 12 bivalents and 1 monovalent; C, triplo-C, 2nd metaphase, 12+13; D, triplo-C, interkinesis showing micronuclei (chromosome count not clear); E, triplo-A, ate 2nd anaphase, 2 laggards from division of unpaired chromosome in 1st division. ×3050.,

(figure 1E). In the second anaphase, after assortment in the heterotypic division, the unpaired chromosome divides so that 13+13 and 12+12 result; or after division at the first anaphase, assortment occurs forming 12+13, 12+13. As before, the unpaired elements often lag during the second anaphase and 2 laggards are seen in one plate or 1 in each (figure 2E). Since 12+13 is more common than 13+13 at the second metaphase, it was surprising to find 1 laggard in each second anaphase plate more commonly than 2 laggards in one plate. Apparently lagging in the second anaphase is more common in the process of *assortment* of the 2 unpaired elements than in the *division* of 1 or, in other words, in the second division assortment is slower than the completion of the process of division. It is evident from the above statement that there is much variation in chromosome behavior during meiosis. While the process tends to follow a certain rule there is no constant behavior of the chromosomes even in material prepared on the same day and from the same anther. Such irregularity seems to be characteristic of meiosis where unpaired chromosomes are present.

The microspore groups or "tetrads" of a simple trisomic may contain 1 or 2 microcytes (figure 1E). Owing to the compact arrangement of the microspores, the counting of microcytes was usually made after much shrinkage had been allowed to occur. The occurrence of micronuclei at interkinesis noted above indicates that some microcytes originate in the heterotype division. From 4.5 percent to 7.6 percent of "tetrads" containing microcytes were counted in 3 different simple trisomic types (table 3). Probably the proportion differs significantly in these types although considerable variation was found in different samples from the same type. In the "tetrads" of double trisomics 3 microcytes were often present.

TABLE 3

*Microcytes in pollen "tetrads" of trisomic tomatoes.*

| NAME | NUMBER OF "TETRADS" EXAMINED | PENTADS AND HEXADS, PERCENT |
|---|---|---|
| Triplo-A | 513 | 4.5 |
| Triplo-B | 426 | 7.1 |
| Triplo-C | 1154 | 7.6 |

## GENERAL CHARACTERISTICS AND NAMING OF TRISOMICS

All the trisomic plants which have appeared either in the $F_1$ progeny of triploids or in the progeny of trisomics show certain general characteris-

tics which serve to distinguish them from diploids. They are slower-growing, and more or less distinct in foliage. They have a tendency to pollen sterility and unfruitfulness which is more marked under field than under greenhouse conditions. Although even simple trisomics are distinguished from diploids by their smaller size up to the time when the first fruits set, later in the season certain of them may exceed diploids in plant size, doubtless on account of their unfruitfulness. Most of the trisomic types are later in flowering, show some difference from diploids in the size or structure of the floral organs and produce fewer viable seeds. These peculiarities are in general more marked in double and triple than in simple trisomics, especially the slower rate of growth, pollen sterility and diminished seed production. In the chromosomal mutants of Matthiola, according to FROST (paper in preparation) hybrid forms are much more fertile than non-hybrid. So in tomato, certain double-trisomic hybrids set seed more freely and were probably more vigorous and more fertile than the corresponding non-hybrids. When the trisomic hybrid type can readily be identified, it is therefore preferable to the non-hybrid for breeding work, but the differences introduced by segregation of genes from hybrid parents increase the difficulty of recognizing extra-chromosome types, especially those containing chromosome fragments.

An attempt has been made to point out some of the most conspicuous features in which certain trisomic types differ from the diploid. Only a beginning has been made in the description of these types. Some of the simple trisomic plant types are by no means easy to distinguish from diploids (see figures 6 and 12). In tobacco the discovery by CLAUSEN and GOODSPEED (1924) of the simple trisomic "Enlarged" was made by the use of a correlation table. So in the tomato, the distinguishing characteristics of certain trisomics, such as rate of growth, number of loculi in the fruit, and foliage color are of a quantitative nature and so variable as to make identification difficult to one unfamiliar with the types, especially where only a small number of plants are observed.

In the naming of trisomics, a modification of the method used by BRIDGES (1921) is adopted here. The capital letters of the alphabet are used to distinguish the individual chromosomes. The haploid number of chromosomes in the tomato is 12 but as yet only nine different types each with 1 whole extra chromosome have been found. Accordingly the letters A to I are used to name these 9 chromosomes. A simple trisomic (25 chromosomes) may then be triplo-A, which means that the A-chromosome is present in triplicate, the remainder being made up of 11 normal pairs. A double trisomic may be triplo-AB, which means that the A- and B-

chromosomes are present in triplicate, the balance consisting of 10 normal pairs.

## DESCRIPTION OF TRISOMIC TYPES

Since the distinguishing characteristics of trisomic types may be masked in hybrid progenies, the non-hybrid progeny of triploid Dwarf Aristocrat was used as the basis for the following descriptions.

FIGURE 3.—Triploid (left) and diploid (right) plants of Dwarf Aristocrat tomato.

The diploid form of Dwarf Aristocrat shows the characteristics of stem and leaf which combine to make the dwarf habit. The stem is short and stiff, giving the plant a rather erect habit until borne down by the weight of the fruit. The leaves are dark green, thick and leathery in texture and deeply rugose, and the leaflets are nearly sessile on the rachis (figures 5 and 6). The style length is about 5 mm and the top of the stigma is

TABLE 4

*Shape and number of loculi ("cells") in fruit of diploid and non-hybrid trisomic tomatoes.*
(For explanation of terms, see figure 4, legend)

| | NUMBER OF FRUITS MEASURED | MEAN E. D. | MEAN $\frac{\text{E. D.}}{\text{L}}$ | MEAN $\frac{\text{L}}{\text{P. D.}}$ | NUMBER OF FRUIT CUT | MEAN NUMBER OF CELLS PER FRUIT | STANDARD DEVIATION OF NUMBER OF CELLS |
|---|---|---|---|---|---|---|---|
| Diploid | 47 | 4.7 | 1.2 | 1.2 | 87 | 4.4 | 1.4 |
| Triplo-A | 9 | 5.4 | 1.4 | 1.4 | 8 | 5.4 | |
| Triplo-C | 4 | 5.2 | 1.5 | 1.7 | 50 | 8.8 | 2.6 |
| Triplo-D | 14 | 3.9 | 1.0 | | 15 | 3.3 | |
| Triplo-G | 6 | 5.2 | 1.5 | 1.4 | 3 | 8.0 | |
| Triplo-H | | | | | 10 | 6.5 | |
| Triplo-I | 7 | 6.2 | 1.3 | 1.3 | 7 | 7.7 | |

usually about level with the apex of the staminal column (figure 21B); this arrangement probably tends to insure self pollination. As a rule pollen is abundantly produced but exceptionally nearly sterile anthers are found. This type set an abundance of fruit in the field without artificial pollination. The color of the fruit is scarlet. Table 4 shows the results of

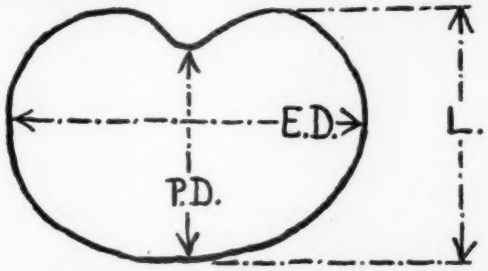


FIGURE 4.—Diagram of fruit. E.D. = equatorial diameter; P.D. = polar diameter; L. = length.

certain measurements of fruits all of which developed in the same season on plants growing in the greenhouse. The meaning of the terms used is shown in figure 4. The values of the ratios of mean equatorial diameter

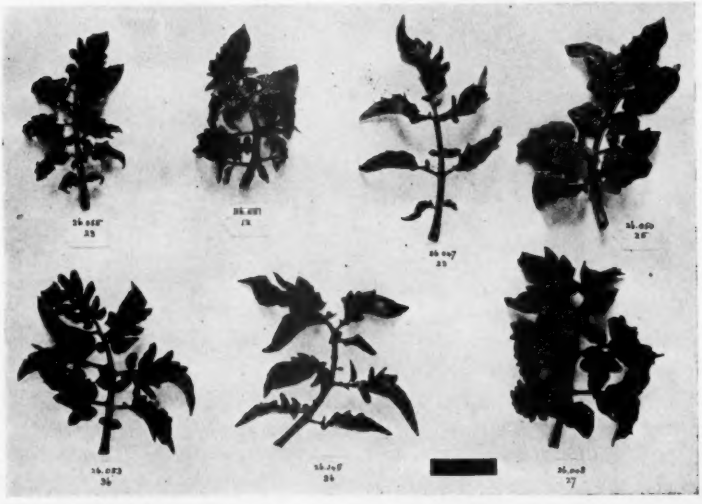

FIGURE 5.—Leaves of diploid and simple-trisomic types of Dwarf Aristocrat (non-hybrid). Triplo-F (26.055.23); triplo-D (26.051.12); triplo-A (26.047.23); triplo-B (26.050.25); triplo-C (26.053.36); diploid (26.048.26); triplo-E (26.048.27).

to length and of length to polar diameter show that the fruit of the diploid is slightly oblate and has shallow polar depressions (figure 25). As a rule it is fairly free from corrugations or longitudinal grooves. The number of loculi or "cells" in the fruit is also shown in table 4, and figure 24D shows a transverse section.

*Simple-trisomic types*

Triplo-A (figures 5, 7 and 8)

Like the other trisomic types, triplo-A is slower-growing than the diploid and is probably intermediate between the more rapidly growing

FIGURE 6.—Diploid non-hybrid (Dwarf Aristocrat), in field.

trisomic types such as triplo-B and the slower types such as triplo-D. The young foliage tends to be paler than that of the diploid. The leaflets appear to be farther apart, are more acuminate and have fewer deep but more shallow irregular incisions and a more undulate margin. The terminal leaflet is often somewhat twisted and the smaller leaflets or foliolules are much reduced in size. The style is remarkably short; the stigma is about 2 mm below the apex of the staminal cone and often malformed, infolded or without a distinct disc (figure 21A). Under field conditions triplo-A has less pollen and more empty grains than the

FIGURE 7.—Triplo-A non-hybrid (Dwarf Aristocrat), in field.

FIGURE 8.—Triplo-A non-hybrid (Dwarf Aristocrat), in greenhouse.

diploid (table 5). On 88 plants of this type not one fruit set in the field without hand pollination. Only 9 fruits were measured; these were distinctly larger and more oblate and had deeper polar depressions and more cells (figure 24C and table 4) than the diploid fruits.

TABLE 5

*Pollen sterility.*

| | IN FIELD | | | IN GREENHOUSE | | |
|---|---|---|---|---|---|---|
| | Number of grains examined | Bad grains (percent) | Bad grains (percent in total) | Number of grains examined | Bad grains (percent) | Bad grains (percent in total) |
| Diploid Dwarf Aristocrat | 225 | 12 | | | | |
| " | 285 | 13 | | | | |
| " | 231 | 9 | 11 | 177 | 3 | 3 |
| Triploid Dwarf Aristocrat | | | | 155 | 75 | |
| " | | | | 108 | 92 | 87 |
| Triploid Dwarf Champion | | | | 84 | 80 | 80 |
| Triplo-A, hybrid | | | | 202 | 14 | |
| " | 307 | 66 | | 202 | 6 | |
| " | 334 | 51 | 58 | 139 | 20 | 8 |
| Triplo-B, hybrid | 272 | 35 | | | | |
| " | 249 | 33 | | | | |
| " | 674 | 30 | 31 | 176 | 26 | 26 |
| Triplo-C, non-hybrid | 383 | 64 | | | | |
| " | 264 | 84 | | | | |
| " | 957 | 54 | | | | |
| " | 337 | 70 | 63 | 119 | 17 | 17 |
| Triplo-D, non-hybrid | 252 | 74 | 74 | 135 | 39 | 39 |
| Triplo-E, non-hybrid | 350 | 25 | 25 | | | |
| Triplo-F, hybrid | 274 | 49 | | | | |
| " | 302 | 26 | 37 | | | |
| Triplo-G, non-hybrid | 83 | 27 | 27 | | | |
| Triplo-H, non-hybrid | 76 | 27 | 27 | | | |
| Triplo-A?, hybrid | | | | 295 | 31 | 31 |

A triplo-A hybrid type of standard habit has been described elsewhere (J. W. LESLEY 1926). It has been shown that the extra chromosome present is associated with the locus of the dwarf-standard pair of genes and that the *Ddd* plant, although standard, shows a suggestion of the dwarf character in the greater thickness and rugosity of the leaflets and the shorter style.

### Triplo-B (figure 9)

This seems to be one of the more rapidly growing simple-trisomic types. The plant has a characteristic pale olive-green color and metallic cast, especially under field conditions; this difference from the diploid color

may be due to the denser coating of trichomes. The leaflets have a greater tendency to downward or inward rolling of the edges, and the rachis of the leaflets has less tendency to curling than with the diploid (figure 5). The spread of the calyx and corolla is smaller and the stamens shorter than in the diploid. An outstanding characteristic is the length of the style, which is about 1 mm greater than that of the diploid; as a rule the

FIGURE 9.—Triplo-B non-hybrid (Dwarf Aristocrat), in field.

stigma projects considerably beyond the relatively short stamens (figure 22). In the field the quantity of pollen, in proportion to the small size of the stamens, is nearly equal to that of the diploid, and the quality (table 5) was superior to that of triplo-A and among the best of the trisomics. Triplo-B plants often set a few fruits in the field and in the greenhouse without hand pollination.

Triplo-C (figure 10)

This type is intermediate among the simple trisomics in rate of growth, or about equal to triplo-A. The foliage, especially in young plants, is somewhat duller green and the stem is thicker and stiffer and the internodes shorter than in the diploid. The leaflets appear closer together (figure 5) and the edges more uprolled. The peduncles are shorter than

FIGURE 10.—Triplo-C non-hybrid (Dwarf Aristocrat), in field.

those of the diploid. The pollen was scanty and probably inferior in quality to triplo-B in field (table 5) but not in greenhouse plantings. The style appears to be especially variable in length but is on the average shorter than that of the diploid. An interesting characteristic of this type is the greatly increased fasciation of the flower. The number of stamens and other floral organs is much increased; table 4 shows that the

number of loculi in the ovary is more variable but the mean is twice that of the diploid. The interior of some fruits (figure 24A) suggests that of the most highly fasciated varieties such as Trophy. In the field 23 plants of this type set no fruit. Under greenhouse conditions and without artificial pollination, the diploid sets some fruits which are as a rule nearly seedless. Unlike other simple-trisomic types triplo-C sets fruit under these conditions quite as readily as the diploid.

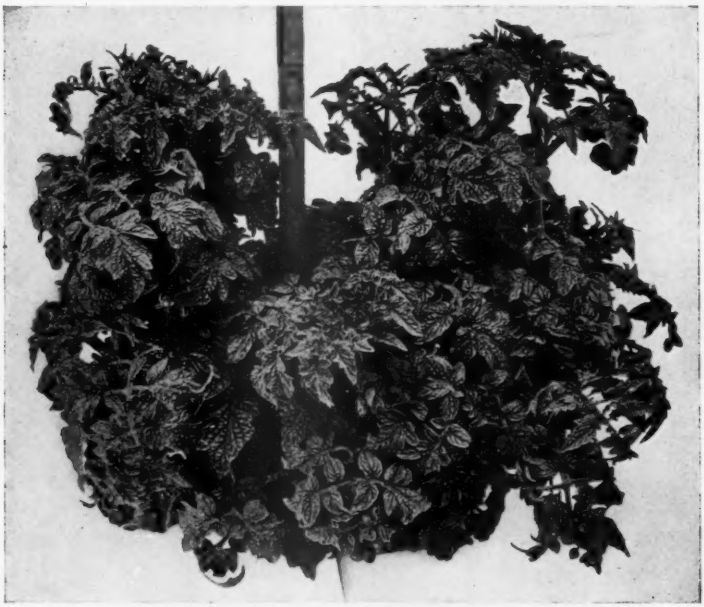

FIGURE 11.—Triplo-D non-hybrid (Dwarf Aristocrat), in field.

### Triplo-D (figure 11)

This type is one of the slower-growing simple trisomics and, especially in the field, is far behind the diploid in plant size, previous to fruit setting. The stem is relatively thin, and the leaflets are smaller, narrower and with more deep incisions than in the diploid (figure 5). Most characteristic of this type is the downward (inward) curling of the rachis and of the rachillae, especially of the terminal leaflet. This feature is more marked under field conditions, where the plant presents a compact, almost cushion-like

appearance. The sepals are longer and more recurved (figure 23B) and the stamens more tapering than in the diploid. The quantity of pollen is only slightly less than in the diploid but the quality is probably much inferior (table 5). The flower shows *less* tendency to fasciation than the diploid, and the fruit has fewer loculi, is smaller and distinctly less oblate in shape (table 4 and figures 24E and 25). In the field among 25 plants of

FIGURE 12.—Triplo-E non-hybrid (Dwarf Aristocrat), in field.

this type one set a single fruit without hand pollination. In the greenhouse, after hand pollination, triplo-D set fruit and viable seed nearly as freely as the diploid.

### Triplo-E (figure 12)

This is one of the relatively fast-growing trisomics and in general most nearly resembles the diploid. The leaves however are darker green

and have shorter petioles and broader, less acuminate leaflets, giving the leaf a more rounded and less spaced appearance (figure 5). The rachis and rachillae, especially of the terminal leaflet, are more curled and in the field the plant forms a compact, almost pillarlike mass of vegetation. The flower and fruit closely resemble the diploid but the ripe fruit seems

FIGURE 13.—Triplo-F hybrid (Dwarf Aristocrat×Globe $F_2$ plant), in field.

to be firmer and contains fewer viable seeds. In the field among some 30 plants of this type, 1 set a single fruit without artificial pollination.

Triplo-F (figure 13)

This type is one of the slower-growing simple trisomics. Young plants are much paler green but later the color tends to approach the normal diploid. The leaflets (figure 5) have fewer deep incisions or lobes but more serration. The surface of the leaves has more fine rugosity and is more velvety than that of the diploid. In the field the pollen appears to be of

relatively good quality (table 5) or about equal to triplo-B. Six field-grown plants set no fruit.

Triplo-G (figure 14)

This is one of the more rapidly growing trisomics, like triplo-B and triplo-E. The stems are weaker and the plant is more decumbent in habit than the triplo-C. The leaflets are narrower and comparatively free from

FIGURE 14.—Triplo-G non-hybrid (Dwarf Aristocrat), in greenhouse.

both lobing and serration. Observation of a few specimens indicates that the fruit is more oblate and has a deeper basin (stylar-end depression) than the diploid.

Triplo-H (figure 15)

Only one plant of this type has appeared. It has fewer foliolules and narrower, more acute leaves than the diploid. The leaflets are almost free from lobing and serrations, giving the foliage a very distinctive appearance. The leaf surface unlike that of triplo-I is as rugose as with the diploid. The flower is probably larger than that of the diploid.

Triplo-I (figure 16)

This type resembles the preceding in having fewer foliolules and less lobing or serration than the diploid. It is distinguished from triplo-H

FIGURE 15.—Triplo-H non-hybrid (Dwarf Aristocrat), in greenhouse.

and from the diploid by its less rugose leaves and by paleness of the red flesh color of the interior of the fruit.

*Double-trisomic types*

Double trisomics are in general even slower-growing than simple trisomics and tend to have less good pollen and less viable seed. The 10

FIGURE 16.—Triplo-I non-hybrid (Dwarf Aristocrat), in greenhouse.

FIGURE 17.—A simple trisomic plant closely resembling triplo-I and perhaps a secondary of it.

non-hybrid double-trisomic plants found belonged to as many different types. This is not surprising since theoretically 9 simple trisomic types may give 36 combination double-trisomic types. Most of the non-hybrid double trisomics were planted in the field. On account of their slow growth and incomplete development, and the small number of plants of each type, adequate descriptions were not obtained. It might be expected that a double trisomic would show a combination of the characteristics of two simple-trisomic types. To some extent, this expectation was fulfilled. Thus one hybrid double trisomic had the curled foliage and small fruit of triplo-D and the pale green color of triplo-F. On selfing both of these types appeared in addition to diploids. One non-hybrid double trisomic was surprisingly like a diploid but had the projecting style and metallic green cast of triplo-B, and like triplo-C it set fruit freely in the greenhouse without pollination. The pollen was very scanty and to obtain progeny it was necessary to backcross to the diploid. The fruits contained but little more seed than the triploid. Among the progeny were triplo-B and triplo-C. A third double-trisomic type clearly suggested

FIGURE 18.—Non-hybrid (Dwarf Aristocrat) "runts"; plants at right and center contained 26 chromosomes in root tips.

triplo-D by its curled foliage and calyx. In other double trisomics the characteristics of simple-trisomic types were not clearly recognized, in some cases perhaps, owing to blending and neutralization of the effects of the extra chromosomes.

A few 26-chromosome plants which originated in the triploid × diploid $F_1$ and were therefore probably double trisomics, were termed "runts" on account of their imperfect development. The shoot failed to form a bud and consisted of a pair of cotyledons and usually one first leaf which was either tube-like or more normal (figure 18). Owing to the incomplete development and lack of distinctive characters, it is not certain whether they belonged to more than one genotype.

### *Triple-trisomic types*

Two 27-chromosome types were found which, from their origin, were presumably triple trisomics. Although extremely slow-growing they were not "runts" but were perfect in development. Two plants of the remarkable type shown in figure 20 have occurred in the non-hybrid $F_1$. This type had almost entire leaves like triplo-H, an abnormal stigma like triplo-A and a fasciated ovary like triplo-C; the anthers contained extremely little pollen. The other type (figure 19) was pale green like triplo-F, and curled foliage like triplo-D and had almost entire leaflets like triplo H.

FIGURE 19.—Non-hybrid (Dwarf Aristocrat) 27-chromosome plant with some characteristics suggestive of triplo-D, triplo-F, and triplo-H.

FIGURE 20.—Non-hybrid (Dwarf Aristocrat) 27-chromosome plant with some characteristics suggestive of triplo-A, triplo-C, and triplo-H.

### *Discussion of trisomic types*

According to BLAKESLEE and BELLING (1924), in Datura 11 or perhaps 12 different primary simple-trisomic types have been identified, each containing 1 extra chromosome of the normal haploid set of 12. DE VRIES and BOEDIJN (1924) report that the Oenothera mutants and the progeny

of *semigigas*-forms of *O. Lamarckiana* and *O. biennis* having 15 chromosomes, fall into 7 groups corresponding to the haploid number of chromosomes in *O. Lamarckiana*. Accordingly if all of the 12 chromosomes of the haploid set in tomato are different in their effect, 12 primary simple-

FIGURE 21.—Styles of non-hybrids (Dwarf Aristocrat). A, triplo-A, short; B, diploid, normal.

FIGURE 22.—Style of triplo-B non-hybrid (Dwarf Aristocrat), long.

trisomic types may be expected to occur in the triploid×diploid $F_1$. Hitherto 9 such types, each with a single apparently whole extra chromosome, have been found. In view of the unequal frequency with which the corresponding types occurred in Datura and of the relatively small number of tomato plants studied, there is reason to think that more simple-trisomic types will be found.

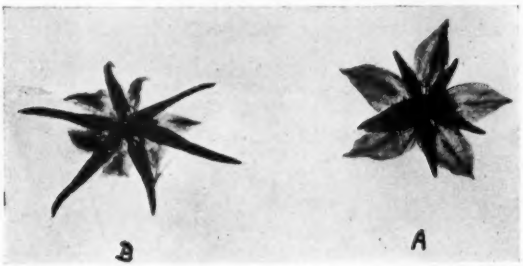


FIGURE 23.—Sepals of non-hybrids (Dwarf Aristocrat). A, diploid, normal; B, triplo-D, long.

For the present, it is preferable to regard these 9 types as homologous with primary mutants of Datura because they originated in the triploid ×diploid $F_1$, the number of simple-trisomic types found is less than the haploid number of chromosomes and the data (table 6) do not justify the conclusion that any of them is rare. Moreover the 9 types are quite distinct in character and only 1 of the 6 types tested gave trisomic ratios

for dwarf. As will be noted later, 2 of them have occurred as mutants in the progeny of other trisomics but in each case the mutant type showed no special resemblance to the parent and belonged to another type pre-

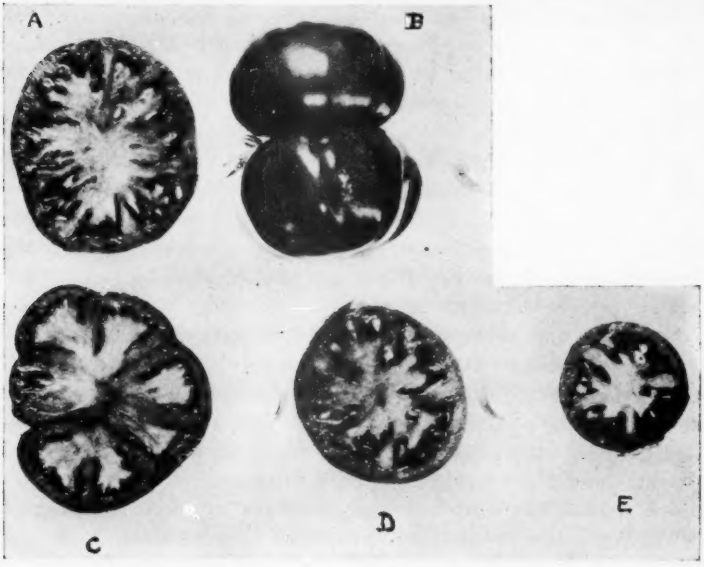


FIGURE 24.—Fruits of non-hybrids (Dwarf Aristocrat). B, triplo-C; transverse sections; A, triplo-C; C, triplo-A; D, diploid; E, triplo-D.

FIGURE 25.—Fruits of non-hybrids (Dwarf Aristocrat). Triplo-G (left); diploid (centre); triplo-D (right).

viously recognized in the $F_1$ from triploid×diploid. Moreover there is some cytological evidence to suggest that these mutations resulted from

lagging of members of a normal chromosome pair. On the other hand one plant (figure 17) in the non-hybrid $F_1$, containing 1 apparently whole extra chromosome, although distinct, resembled triplo-I very closely. Whether this represents another primary type or whether it is a secondary or merely a result of gene mutation cannot as yet be stated. That chromosome fragmentation occurs is shown by the discovery by M. M. LESLEY (unpublished data) of a plant in the non-hybrid $F_1$ containing 1 whole extra chromosome and an additional fragment, and of other fragment-containing plants in the progeny of trisomics.

It is concluded that the characteristics of each of the 9 simple-trisomic types of tomato are due to the presence of a whole extra chromosome and it is inferred that at least 9 of the 12 chromosomes are different in effect. The fact that no simple-trisomic type has been found which is identical with the diploid, is consistent with the assumption that all of the 12 chromosomes are different.

In haplo-IV and in triplo-IV *Drosophila melanogaster*, BRIDGES (1921) observed a change in the grade of mutant characters due to the IV-chromosome genes, demonstrating clearly the relation between these characters and this particular chromosome. But he also found a change in the grade of mutant characters, the major genes for which were not in the IV-chromosome. This indicated the presence in the IV-chromosome of genes modifying these characters. Similarly in Datura, a single gene difference determines purple and white flower color but BLAKESLEE (1921a) found that in a homozygous purple race, the intensity of color differed widely in the trisomic mutants. Thus Glossy was darker and Cocklebur lighter than the normal. Since neither of these types proved to contain the extra chromosome with the major gene for purple color, the differences indicate the presence of modifying genes in different chromosomes. BRIDGES (1922) has advanced a hypothesis which appears to harmonize admirably with the known facts relating to the distribution and number of major and modifying genes. According to that author, a change in the grade of a character occurs if the extra chromosome present is unbalanced for the genes affecting that character. Now an outstanding feature of the trisomic types of tomato is that many of their distinctive characteristics strongly suggest those which are known to depend on gene differences. Thus the almost entire leaflets of the triplo-H suggest the potato-leaf character, which is a simple recessive to cut leaf. The pale green color of triplo-F is suggestive of the "yellow" foliage character, which according to PRICE and DRINKARD (1908) is a simple recessive to "green" in certain varietal crosses. Differences in number of loculi in the fruit similar to

those seen in triplo-D and triplo-C have been shown by PRICE and DRINKARD (1908) and by WARREN (1924) to depend on gene differences. Compared with the diploid, fruit size is smaller in triplo-D and probably larger in triplo-A. According to LINDSTROM (1926), the evidence clearly indicates that hereditary factors for fruit size and fruit color depend on the same chromosomal mechanism.

Just such a similarity between the effects due to gene mutations and to the presence of extra chromosomes is to be expected according to BRIDGES' hypothesis of gene balance. Moreover, in tomato, a change in the grade of a given characteristic commonly occurs in more than one trisomic type, and in some cases the change is in opposite directions. For example triplo-H tends to have leaflets more nearly entire and triplo-D more dissected than the diploid. Triplo-F is paler green, and triplo-E darker green than the diploid. Triplo-D has fewer loculi in the fruit and triplo-C more than the diploid. This is in accordance with the known fact that number of loculi is not dependent on a single gene pair. Probably this is also true of foliage color and shape of leaflets. Where other evidence suggests that the number of genes influencing a characteristic is relatively small, it is simpler to suppose that the change of grade is due to the presence in the extra chromosome of a single gene influencing the grade of that character. In the case of fruit size, however, which undoubtedly depends on a large number of genes, BRIDGES' conception of gene balance is especially appropriate. Thus the small fruit size of triplo-D may well be due to an excess of modifiers in the minus direction in the internal gene balance of the D-chromosome.

Little is known of the genetic basis of growth rate but it is probably not genetically simple. Since trisomic types differ in rate of growth, it appears that the internal balance of genes affecting growth differs in different chromosomes. Thus the fact that triplo-B is nearer to the diploid in growth rate than triplo-D suggests that the internal balance of genes affecting growth is nearer to the normal (diploid) in the B-chromosome than in the D-chromosome. It seems to be a general rule, to which the tomato conforms, that *no* trisomic type is quicker-growing than the diploid, as if unbalance of chromosome number were never associated with acceleration of growth. In terms of + and − modifiers there is not an excess of plus modifiers in any of the chromosomes. Hence if genes affecting growth are involved, they cannot be adequately classified merely as + and − modifiers but must have more complex relations, perhaps of a complementary nature.

Attention has already been called to the very abnormal development of certain plants, probably double trisomics, which were termed "runts," and to the perfect development of other plants, probably triple trisomics. Clearly these facts cannot be explained on the basis of unbalance in chromosome number. According to BRIDGES' theory of gene balance, however, 2 extra chromosomes with a gene unbalance of the same kind would cause a greater disturbance than 3 extra chromosomes which tend to neutralize one another.

That some attributes of trisomics differ from any gene mutation known in tomato may mean that such mutations have not occurred or have not been preserved. It is possible that some effects due to extra chromosomes, such as curling of foliage and extreme imperfection of development, may not be attainable by any combination of genes in a diploid plant.

## FREQUENCY DISTRIBUTION OF OCCURRENCE OF TRISOMIC TYPES

The frequency distribution of the simple trisomic types is shown in table 6. The data do not suggest the great predominance of any one type, and and the absence of three expected types is not surprising considering the small number of plants. Triplo-D, one of the least vigorous types, occurred most frequently, and triplo-H, which is probably one of the more vigorous, occurred least frequently. The chromosome number was not determined in every individual but in all determined cases without exception the same plant type was found to have the same number of chromosomes.

TABLE 6

*Frequency of occurrence of simple-trisomic types in triploid×diploid $F_1$.*

| | TRIPLO- | | | | | | | | | TOTAL NUMBER OF $F_1$ PLANTS |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | |
| Frequencies observed | 5 | 4 | 5 | 6 | 4 | 3 | 5 | 1 | 3 | 91 |

The far more complete data of BLAKESLEE (1924) show that in the progeny of triploid Datura, the simple-trisomic types Echinus and Rolled occur less commonly than Globe and Buckling. In Oenothera DE VRIES and BOEDIJN (1924) found that *cana* occurred more often than *lata* among the 15-chromosome progeny of semigigas (triploid)×*velutina* (diploid). As already noted the 10 double- and 2 triple-trisomic tomato plants belonged to as many distinct types.

## TRANSMISSION OF EXTRA CHROMOSOMES

If the extra chromosome of a simple trisomic is distributed to half of the gametes in meiosis, with equal viability of gametes and zygotes and

with random mating, a simple-trisomic plant, if fertile, on selfing would give 25 percent simple-tetrasomic, 50 percent simple-trisomic and 25 percent diploid progeny; on backcrossing to a diploid, 50 percent simple trisomics and 50 percent diploids would result. That this expectation was not fulfilled is shown by the data from selfing and backcrossing six of the trisomic types shown in table 7.

Several reasons may be suggested for the failure of trisomic plants to transmit the extra chromosomes through the pollen to the full extent. In the pollen mother cells of a simple trisomic the frequent lagging of the unpaired chromosome and the presence of microcytes show that less than

TABLE 7

*Transmission of extra chromosomes by trisomic parents.*

| | POPULATION NUMBER | NUMBER OF PLANTS | GERMINATION PERCENT | PERCENT TRIPLO- | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A | B | C | D | E | F |
| Triplo-A hybrid selfed | 25.063 | 47 | | 23 | | | | | |
| Triplo-A hybrid×diploid | 25.065 | 88 | | 24 | | | | 1 | |
| Do. | 26.046 | 72 | 80 | 25 | | | | | |
| Triplo-A hybrid×Dwarf Champion diploid | 25.064 | 89 | | 18 | | | | | |
| Do. | 26.047 | 67 | 81 | 33 | | | | | |
| Diploid×triplo-A hybrid | 25.062 | 43 | 80 | | | | | | |
| Triplo-B hybrid×diploid | 26.050 | 83 | 93 | | 27 | | | | |
| Diploid×triplo-B hybrid | 26.049 | 90 | 100 | | 23 | 1 | | | |
| Triplo-B hybrid selfed | 26.059 | 92 | 96 | | 37 | | | | |
| Triplo-C non-hybrid selfed | 26.052 | 61 | 67 | | | 7 | | | |
| Triplo-C hybrid selfed | 25.075 | 31 | | | | 26 | | | |
| Do. | 26.056 | 54 | 75 | | | 20 | | | |
| Diploid×triplo-C non-hybrid | 26.053 | 52 | 84 | | | 10 | | | |
| Triplo-D non-hybrid selfed | 26.051 | 64 | 73 | | | | 29 | | |
| Triplo-E non-hybrid selfed | 25.074 | 33 | | | | | | | |
| Do. | 26.048 | 70 | 89 | | | | | 16 | |
| Triplo-E hybrid selfed | 25.072 | 53 | | | | | | 21 | |
| Do. | 26.057 | 24 | 89 | | | | | 17 | |
| Triplo-DF hybrid selfed | 26.055 | 34 | 57 | | | | 9 | | 24 |
| Triplo-C?×diploid | 25.073 | | | | 18 | | | | |

half the microspores of the tetrads contain the extra chromosome. But microcyte formation alone does not account for the deficiency in pollen transmission. For triplo-A, which failed entirely to transmit the extra chromosome through the pollen (table 7), had only 4.5 percent of tetrads containing microcytes (table 3), and triplo-B, which gave 23 percent pollen

transmission, had a *greater* proportion of tetrads containing microcytes than triplo-A. The pollen sterility of all trisomic types probably contributes to the low pollen transmission. Since the unbalance in chromosome number of 13-chromosome pollen is as great as that of a simple-tetrasomic plant, the proportion of inviable grains is probably greater among 13-chromosome than among 12-chromosome grains. Triplo-B had fewer "bad" pollen grains than triplo-A or triplo-C and correspondingly transmitted the extra chromosome more readily through the pollen. Triplo-C had less "bad" pollen than triplo-A and higher pollen transmission. Another possible cause of defective pollen transmission is competition between the pollen tubes. Thus BUCHHOLZ and BLAKESLEE (1922) found that, in the Globe mutant of Datura, tubes from pollen with the extra chromosome are slower-growing than those of normal pollen. Consequently the application of an excess of pollen may lead to selective fertilization.

While spore sterility may thus account for defective pollen transmission, it probably does not account for defective transmission through the eggs. BUCHHOLZ and BLAKESLEE (1922) found a larger proportion of aborted ovules from Globe×normal than from normal×normal. Some of these ovules apparently were fertilized and had suffered in competition for food with diploids. In tomato the relatively small number of viable seeds in the fruit of triploids and of trisomic types suggests that the small inviable seeds are those with extra chromosomes. Such zygotic sterility may well account for the deficiency of ovule transmission.

The frequency of transmission of the extra chromosome varied considerably in progenies of similar origin. Thus 26.048 (table 7), the progeny of triplo-E selfed, contained 16 percent, but 25.074, a small population from different seed of the same parentage, contained no trisomic plants. It should be mentioned that these 2 progenies were grown under somewhat different cultural conditions. Again 26.052, from non-hybrid triplo-C selfed, contained 7 percent trisomics, but 26.056 and 25.075, from hybrid triplo-C selfed and from the same seed sample, contained respectively 20 percent and 26 percent triplo-C. These data suggest that the hybrid triplo-C parent transmits the extra chromosome somewhat more readily than the non-hybrid. This may be due in part to the superior germination of the hybrid seed (26.056) compared with the non-hybrid (26.052). According to FROST (1919) it is probable that with the mutant forms of Matthiola, higher frequency of transmission of the extra chromosome is associated with higher percentage of seed germination within the same non-hybrid race.

The frequency of transmission seems to differ significantly in different trisomic types. In hybrids, it ranged from 37 percent from selfing in triplo-B to 20 percent in triplo-C and in non-hybrids from 29 percent in triplo-D to 7 percent in triplo-C. In Matthiola, FROST and MANN (1924) report that in selfing 4 different mutant types, the frequency of transmission by non-hybrids has varied from 25 to 50 percent; later results (FROST, unpublished data) suggest that $F_1$ hybrid forms tend to give a higher frequency than non-hybrid forms of the same mutant types.

In Datura, the frequency of transmission of the extra chromosome was found to be much lower through the pollen than through the eggs. Thus BLAKESLEE (1921b) reports that the Globe mutant gave about 25 percent transmission through the eggs but only about 3 percent through the pollen. Again CLAUSEN and GOODSPEED (1924) found 35.5 percent of ovule transmission but only 3.4 percent of pollen transmission of the trisomic form "Enlarged" of *Nicotiana tabacum*. In Matthiola on the other hand, FROST and MANN (1924) report that the chromosome or chromosome fragment present in the mutant Slender has been transmitted to nearly 20 percent of the progeny through the pollen. In tomato there seems to be little or no transmission of the extra chromosome through the pollen of triplo-A. For, a small population from diploid×triplo-A contained no trisomic plants and the percentage of trisomics in the progeny of triplo-A hybrid selfed was practically the same as that of triplo-A hybrid×diploids. On the other hand triplo-C transmitted the extra chromosome through the pollen to 10 percent of its progeny. A striking contrast with the results from Datura and Nicotiana was found in the case of triplo-B. Diploid×triplo-B hybrid (26.049, table 7) gave 23 percent triplo-B. The relatively little pollen sterility compared with other simple trisomic types of tomato and the relatively high seed germination in 26.049 are both likely to favour high frequency of transmission. When used as seed parent the same hybrid trisomic gave 27 percent triplo-B or only slightly more than when used as pollen parent. When selfed this plant gave 37 percent triplo-B, which agrees fairly closely with the expectation assuming independence of the processes of transmission through eggs and pollen.

Defective transmission of the extra chromosome, especially through the pollen accounts in part for the scarcity of tetrasomics. Furthermore tetrasomic zygotes are likely to be even less vigorous than trisomics and less able to survive competition with more balanced zygotes. No tetrasomic plants have been found in the progeny from selfing of triplo-A, triplo-C or triplo-E. From the high percentage of pollen transmission

in triplo-B, tetrasomics might be expected in its progeny from selfing. Two remarkably slow-growing plants originated in that way. One of these failed to develop a normal shoot but several adventitious buds developed on the leaves and the flowers resembled triplo-B. Several fruits set from self-pollination. The other plant was normal in development but also far slower-growing than its trisomic sibs. Similarly in the progeny of triplo-D selfed, a single "runt" appeared. The chromosome number of these plants has not yet been determined but they are most probably tetrasomics.

## MUTATIONS

No chromosomal mutations other than triploids have been observed by the writer in commercial plantings or in the progeny of plants which were known to be diploid. Two mutant trisomics have occurred, however, in the progeny of trisomic plants. One population (25.065, table 7) from triplo-A hybrid×diploid Dwarf Aristocrat contained 63 diploid, 24 triploid-A and in addition 1 triplo-E plant which must be considered as a mutant. A single mutant plant of triplo-C occurred among the progeny of diploid Dwarf Aristocrat×triplo-B hybrid (26.049, table 7). In simple trisomics the occasional presence of supernumerary laggards in the pollen mother cells points to irregular behavior of a normal pair of chromosomes and not merely of the extra chromosome. In diploids the occurrence of microcytes in the tetrads also suggests irregularities of chromosome distribution. As these irregularities seem to be more frequent in trisomics than in diploids, on the whole it is probable that the mutants resulted from a disturbance of maturation division in the trisomic parents. Since trisomic mutants tend to be unfruitful, some of the "he" plants or "fruitless hybrids" of the tomato grower may be of this nature. Since, however, these mutants have not been observed in the progeny of diploids and are likely to be crowded out in the seedbed probably the "he" plants are usually either triploids or the result of other causes such as mosaic disease.

## TRISOMIC INHERITANCE AND LINKAGE

A modification of the Mendelian ratios should occur on breeding from a trisomic plant which is heterozygous for genes whose loci are in the extra chromosomes present. BLAKESLEE and FARNHAM (1923) have shown that the Poinsettia mutant of Datura gives trisomic ratios for flower color, and they conclude that the extra chromosome present contains the locus of the allelomorphic genes which determine purple or white flowers.

Trisomic inheritance of the dwarf character in the tomato has been reported by the present writer (1926). The A-chromosome was found to contain the locus of the genes which determine standard and dwarf. As table 8 shows, 5 other simple-trisomic types heterozygous for the standard-dwarf pair of genes have given apparent disomic ratios for dwarf and standard in accordance with expectation. This is also true of the progeny of C86-8 which belongs to a simple-trisomic type not identified.

Triplo-B, triplo-C and triplo-E hybrids and C86–8, all heterozygous for the pair of genes for cut-leaf and potato-leaf, gave disomic ratios for this pair of characters.

The non-yellow skin color of the fruit is due to the recessive member of a single pair of genes *Yy*. When flesh color is red, *yy* plants have crimson and *Yy* or *YY* plants scarlet fruit. Triplo-A plants gave approximately disomic ratios for skin color, which is in accordance with expectation, since dwarf and non-yellow skin depend on genes which are not linked. The data from other simple- or double-trisomic plants heterozygous for skin-color genes indicate that the locus of this pair of allelomorphs is probably not in the B, C,D, E or F chromosomes, since the ratios among the diploid progeny appear nearer to 3:1 than to the 8:1 ratio which would occur in trisomic inheritance.

A study of the combination of characteristics present in the simple-trisomic types may throw light on the distribution of the genes in the different chromosomes. The simplest case is that in which certain characteristics are peculiar to one only of the simple-trisomic types. For those characteristics, if due to genes, must depend either on a single gene or on linked genes present in that particular chromosome. From the preceding account of the trisomic types, however, it is evident that a change in the grade of a characteristic is usually not confined to one trisomic type. In tomato the I-chromosome probably contains genes which modify flesh color of fruit. Since triplo-I has nearly entire leaves, it may be inferred that, in this case, flesh color of fruit and leaf shape depend on the same gene or on genes which are linked. Again the characteristics of triplo-D suggest that certain genes for size and number of loculi in the fruit are linked. From breeding and correlation studies LINDSTROM (1926) has found evidence of linkage between the genes for dwarf habit and fruit size. Since the A-chromosome contains the locus of the gene for dwarf, triplo-A should accordingly differ from the diploid in size of fruit. The few fruits observed seemed to be distinctly larger than the diploid but more data are required to establish this. The relatively small size of seedless fruits and of the few-seeded fruits regularly produced by the

TABLE 8[6]

*Inheritance of habit, leaf form and skin color of fruit.*

*(D = standard, d = dwarf habit; C = cut, c = potato leaf; Y = yellow, y = non-yellow skin of fruit.)*

| | HABIT AND LEAF FORM | | | | | | | | SKIN COLOR OF FRUIT | | | | THEORETICAL MONOHYBRID RATIOS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | Disomic inheritance | Trisomic inheritance | | | |
| | Diploid progeny | | | | Trisomic progeny | | | | Diploid progeny | | Trisomic progeny | | Diploid and trisomic progeny | Diploid progeny | | Trisomic progeny | |
| | *DC* | *De* | *dC* | *de* | *DC* | *De* | *dC* | *de* | *Y* | *y* | *Y* | *y* | *D*:*d*, *C*:*c* or *Y*:*y* | *D*:*d* | *C*:*c Y*:*y* | *D*:*d* | *C*:*c Y*:*y* |
| Triplo-A hybrid selfed | 60 | | 56 | | 8 | | 3 | | 17 | 11 | | | 3:1 | 5:4 | 8:1 | 7:2 | all dominants |
| Diploid × triplo-A hybrid | 13 | | 30 | | | | | | 18 | 18 | | | 1:1 | 1:2 | 2:1 | 2:1 | do |
| Triplo-B hybrid selfed | 54 | 7 | 17 | 7 | 15 | 9 | 6 | 5 | 54 | 11 | 5 | 1 | 3:1 | 5:4 | 8:1 | 7:2 | do |
| Triplo-B hybrid × diploid | 37 | | 24 | | 15 | | 7 | | | | | | 1:1 | 1:2 | 2:1 | 2:1 | do |
| Diploid × triplo-B hybrid | 37 | | 32 | | 6 | | 15 | | | | | | 1:1 | 1:2 | 2:1 | 2:1 | do |
| Triplo-C hybrid selfed | 35 | 19 | 12 | 9 | 7 | 3 | 4 | | 40 | 14 | | | 3:1 | 5:4 | 8:1 | 7:2 | do |
| Triplo-E hybrid selfed | 41 | 11 | 14 | 8 | 10 | 2 | 1 | | 29 | 11 | | | 3:1 | 5:4 | 8:1 | 7:2 | do |
| Triplo-DF hybrid selfed | 17 | | 5 | | 11 | | 1 | | 14 | 8 | | | 3:1 | 5:4 | 8:1 | 7:2 | do |
| Simple trisomic hybrids of unknown type: C86-2 selfed | 38 | 9 | 9 | 5 | 5 | 2 | | | 26 | 13 | | | 3:1 | 5:4 | 8:1 | 7:2 | do |
| C86-7 selfed | 23 | 13 | 7 | 4 | | | | | 19 | 7 | | | 3:1 | 5:4 | 8:1 | 7:2 | do |
| C86-8 selfed | 33 | 9 | 7 | 7 | 5 | 2 | 2 | 2 | 42 | 12 | | | 3:1 | 5:4 | 8:1 | 7:2 | do |

[6] The writer is indebted to Mr. C. W. HANEY, Division of Genetics, UNIVERSITY OF CALIFORNIA, for part of the observations contained in this table.

triploid, point to some degree of correlation between fruit size and seed production in tomato. If so, comparison of fruit size between trisomic types with few seeds such as non-hybrid triplo-A and the diploid is more difficult. CRANE (1915) has found some evidence of linkage between the genes for simple inflorescence and short, that is, oblate, fruit. MACARTHUR (1926) has shown that simple inflorescence and dwarf are linked. Hence triplo-A, which contains the locus of dwarf, should also contain factors modifying length of fruit. The scanty data available (table 4) suggest that this is in fact the case, since in triplo-A, ED/L is 1.44 as compared with 1.25 in the diploid. LINDSTROM (1926) has found good evidence of linkage between *Vy*, the genes for skin color of fruit, and certain major factors determining size of fruit. Hence the simple-trisomic type with the extra chromosome containing the locus of *Vy* should also contain major genes affecting fruit size. As noted above a type giving trisomic ratios for skin color of fruit has not yet been found.

## CONCLUSION

The present paper represents only a beginning of the analysis of the progeny of triploid tomatoes. In particular much more light can be thrown on the composition of the individual chromosomes by a more detailed study of the types and by hybridization. The occurrence of chromosome fragmentation is of special interest and may also throw light on the distribution of the genes and on the problems of size and number of chromosomes.

The foregoing data appear to be wholly consistent with the chromosome theory of heredity. In infectious disease certain conditions, defined by ROBERT KOCH in 1882, are required to prove the causal nature of the infecting organism. A rather close analogy may be drawn between such a pathogenic organism and a chromosome. Extra chromosomes occurred invariably and exclusively in plants with certain variant characters, thus conforming to two of the famous canons. As in the case of obligate parasites the chromosomes have not been grown in culture apart from the host as the third canon requires, but they are transmitted to the progeny and reproduce identical symptoms. The simplest conclusion is that the relation between the extra chromosome and the characteristics of a trisomic plant is causal. To hypothecate an unknown underlying cause of which an extra chromosome is merely a symptom would seem to be gratuitous.

The occurrence of trisomic ratios in the progeny of a hybrid trisomic tomato plant is a further confirmation of the association between chromo-

some and gene. Evidently in the tomato unbalance in terms of chromosome number is an important factor influencing growth and viability. The effects of extra chromosomes on development are such as might result from differences in internal gene balance in different chromosomes, as defined by Bridges, and at present the most reasonable hypothesis is that certain of the effects associated with the presence of extra chromosomes are due to a change in the balance of the genes.

The visible effects caused by a single whole extra chromosome are in some cases small compared with those due to single gene differences. For example the effect of the E-chromosome in triplo-E is less striking than that of the gene causing potato leaf. On the other hand the effect of the D-chromosome in triplo-D is probably greater than that of certain genes. Indeed from its appearance, triplo-D would readily pass as a distinct variety, but owing to its partial sterility and genetic inconstancy, so characteristic of trisomic types, it can scarcely be of importance in the evolution of new varieties.

## SUMMARY

1. Triploid plants have been found in 3 varieties of tomato. They probably originated from the fusion of diploid and haploid gametes.

2. In the $F_1$ from triploid$\times$diploid, chromosome number did not have a binomial distribution but ranged from 24, the diploid somatic number, to 27, with the mode at 25 chromosomes. Degree of unbalance in chromosome number is therefore an important factor influencing viability.

3. The smaller seeds from triploid$\times$diploid gave more 26- and 27-chromosome plants than the larger seeds.

4. From diploid$\times$triploid, only 2 plants were obtained, both of which were diploid.

5. In meiosis in the pollen mother cells of a simple trisomic, the unpaired chromosome is not constant in its behavior. At diakinesis it may either form a trisome or lie separate. It frequently lags in the first and second anaphase. In the heterotype division it may undergo either assortment or division, more often the former. Correspondingly in the homotype division either division or assortment takes place, and probably assortment requires more time than completion of the process of division.

6. The proportion of tetrads containing microcytes varied from 4.5 percent to 7.6 percent in different simple-trisomic types. The presence of micronuclei at interkinesis in the pollen mother cells suggests that microcyte formation sometimes begins at that stage.

7. In general, compared with the diploid, simple-trisomic types are slower-growing and have a greater tendency to pollen sterility and unfruitfulness in the field. These differences are accentuated in double- and triple-trisomic types.

8. From the haploid number of chromosomes, 12 different simple-trisomic types are expected. Of these, 9 have been identified. At least 9 of the 12 chromosomes of the haploid set are therefore different in their effect on development.

9. The 9 simple-trisomic types are "primaries" in the terminology proposed by BLAKESLEE and are due to the presence of a whole extra chromosome of the normal set. Types containing fragments also occur.

10. No variation in chromosome number was found among individuals of the same type, when examined, they invariably proved to have the same number of chromosomes except in the case of tetraploid islands (p. 4).

11. Each of the simple-trisomic types has a distinctive combination of characteristics. In several cases two or more types differ from the diploid in the same characteristic but to a different degree and in different directions.

12. The double- and triple-trisomic types have, in some cases, combinations of the characteristics of simple-trisomic types.

13. Some of the effects of an extra chromosome are due to modifying genes present in it. Where the number of genes affecting a characteristic is large, the effects of an extra chromosome are probably the result of an internal gene balance of the extra chromosome, different from that of the normal chromosome complement.

14. Certain double trisomics are more abnormal in development than certain triple trisomics. Two extra chromosomes with an internal gene balance in the same direction may cause a greater disturbance of development than three extra chromosomes which tend to neutralize one another.

15. Among 35 simple-trisomic plants of 9 different types, no type predominated greatly in number of individuals.

16. The extra chromosome is transmitted to less than the expected proportion of the progeny. The observed proportion from selfing varied with different extra chromosomes. In two simple trisomic types the extra chromosome was transmitted through the pollen almost as frequently as through the eggs. Defective transmission through the pollen is in part due to irregularities in sporogenesis and in part perhaps to pollen sterility and selective fertilization. Defective transmission through the eggs is probably due, in part, to zygote sterility.

17. Tetrasomics probably occur, although rarely, in the progeny of certain simple trisomics.

18. Simple-trisomic types sometimes occur as mutants. In pollen mother cells of trisomics the occasional lagging of members of a normal pair and the occasional presence of microcytes in the tetrads of a diploid suggest how these mutants may arise.

19. Trisomic inheritance of the dwarf character has been found and the simple-trisomic type whose extra chromosome contains the locus of dwarf has been identified.

20. Evidence of linkage between genes may be obtained from a knowledge of the combinations of characteristics occurring in simple-trisomic types, especially when this information is supplemented by results of correlation studies and breeding experiments.

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# FIVE AUTOSOMAL MUTANTS IN *DROSOPHILA HYDEI*[1]

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## INTRODUCTION

A description of *Drosophila hydei* Sturtevant is to be found in STURTEVANT'S monograph on the North American species of Drosophila (1921). It is of particular interest because of its very close resemblance to *D. repleta* and *D. mulleri* and because it is one of the few very common species of the genus. Sex-linked mutants have been described by CLAUSEN (1923) and SPENCER (1927). An autosomal recessive eye color, scarlet, was found by HYDE (1915), and the same author (1922) has reported another mutant, eyeless, a recessive linked to scarlet with about 25 percent of crossing over. The present paper deals with the origin, description, and linkage relations of five new autosomal recessive mutants, red, orange, triangle, facet, and extra-scutellars. Unfortunately HYDE'S scarlet and eyeless could not be used in linkage studies as they had been lost before the finding of the mutants here described.

## RED (*r*), A RECESSIVE EYE COLOR

In a large stock bottle of *D. hydei*, out of a thousand or more flies examined on March 3rd, 1926, several were found with a red eye color. Breeding tests have shown this mutant to be an autosomal recessive of fair viability. The eye color, particularly in the newly emerged fly is a bright red

[1] This work was done at the OHIO STATE LAKE LABORATORY, Put-in-Bay, Ohio and the Biology Laboratory, COLLEGE OF WOOSTER, Wooster, Ohio. I wish to thank Doctors R. C. OSBURN and W. M. BARROWS of OHIO STATE UNIVERSITY for their interest in and encouragement of the work and Doctor R. E. COLLINS of the UNIVERSITY OF CALIFORNIA for furnishing stocks of the white and vermilion mutants of *D. hydei*.

in contrast to the dark dull brown of the wild type, and resembles closely the wild type eye color of *D. melanogaster*. This color darkens considerably with age and approaches more nearly the wild type, but in most cultures can be separated without difficulty even when the flies are old. The description of HYDE'S scarlet indicates that it was somewhat more brilliant than red. The stock in which this mutant arose was derived from a wild stock collected in a fruit cellar in Wooster, Ohio, and this wild stock will be referred to as Wooster stock.

### ORANGE (*o*), A RECESSIVE EYE COLOR

On May 10th, 1926, among several hundred flies removed from a large stock bottle of Wooster stock two females were found having an orange eye color, strikingly different from the wild type. These females were each mated to normal-appearing males from the same bottle. One of these cultures gave all wild type while in the other approximately half the flies were orange eyed. These results showed that orange was an autosomal recessive and that the male in the second culture had been heterozygous for the orange gene. The excellent viability and ease of classification of orange make it especially valuable for linkage studies. This eye color is somewhat like peach of *D. melanogaster* and when compared with charts in Ridgway (1912) is between the colors listed by him as Mars Orange and Burnt Sienna. The color darkens with age and there is little or no shadow in the eye.

Preliminary breeding tests showed that red and orange were linked. A double recessive stock was established. This eye color was easily separable from both red and orange and cultures were then made up of red orange males to females with orange in one chromosome and red in the other. The crossover data from these are given in table 1. Of 3089 flies, 207, or 6.7 percent, were crossovers. The data from the reciprocal type of cross in which red and orange entered in the same chromosome are given in table 2. From 1028 flies, 66 were crossovers, giving a percentage of 6.42. On combining the data out of a total of 4117 flies 273 or 6.63 percent were crossovers. The tentative loci of these mutants are therefore placed as follows: red (0.0) and orange (6.6) in the second linkage group. (This is called the second linkage group as HYDE'S two linked autosomals were not available for testing linkage relations to the genes reported here.)

### TRIANGLE (*l*), A RECESSIVE WING VEIN MUTANT

On January 14th, 1927, in counting flies from culture 827 of the red mutant five normal males, eight normal females, and five males and eight

females showing a slight variation in wing venation were found. When culture 682 from which 827 had been derived was examined, it was found to contain a few flies with the same variation. This culture was made up on November 13th, 1926, and it is probable that the mutant had arisen in the red stock previous to this date.

The character expression of triangle varies from a short extra cross-vein connecting the costal and second veins near the posterior termination of the latter, to two or three of these short cross-veins or a triangular deltoid area formed by the confluence of the costal and second veins for a short distance near the posterior termination of the latter. The character resembles the triangle and confluent mutants in *D. virilis* described by METZ, MOSES and MASON (1923) but differs in being a recessive. In cultures homozygous for triangle many flies show the character only in one wing and others are entirely normal in appearance. Sometimes only a few flies show the character; this is particularly true if the culture is crowded. For this reason triangle is of little value in linkage studies.

As triangle arose in a red stock it was easy to test the two mutants for linkage. It was found that triangle was linked to red and orange. A red orange triangle stock has been made up and the crossover data thus far secured indicate that triangle crosses over freely with the other two loci. However, its extreme variability in the number of normal overlaps of homozygous triangle flies makes it difficult to determine with any degree of accuracy its position relative to red and orange.

The author has found a recessive mutant in *D. melanogaster* (unpublished) which very often resembles triangle. It is interesting to note that this recessive triangle in *D. melanogaster* lies in the third chromosome linked to scarlet and peach, two eye colors which are rather closely linked and which resemble the closely linked red and orange mutants of *D. hydei*. The linkage of these three similar mutations in the two speces is some indication that they are really parallel mutations.

### FACET (*f*), A RECESSIVE EYE TEXTURE MUTANT CAUSING STERILITY IN MALES

In examining flies from culture 969, a stock of the sex-linked mutants Notched-vermilion-bobbed, on February 13th, 1927, one male and two females, not Notched, were found having the hairs on the eyes slightly disarranged and many of the facets rounded up so as to give an appearance of glittering points of light reflected from the surface of the eye. An examination of the parent Notched-vermilion-bobbed stock made up on December 12th, 1926, revealed one such facet female and it is likely that the mutation occurred previous to this in the Notched-vermilion-bobbed

stock. Examination of other Notched-vermilion-bobbed cultures showed the presence of facet in them. The presence of vermilion in the culture probably accounts for the detection of this mutant as the facet character shows up more strikingly against the bright vermilion background. However, the character is readily classified in the presence of any of the mutant eye colors or with the wild type eye color.

TABLE 1

*Linkage of Red and Orange. Red Orange* $\frac{ro}{ro}$ *males* $\times$ $\frac{rO}{Ro}$ *females.*

| CULTURE NUMBER | NON CROSSOVERS | | | | CROSSOVERS | | | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| | Red ♂ | Red ♀ | Orange ♂ | Orange ♀ | Red Orange ♂ | Red Orange ♀ | Mahog-any ♂ | Mahog-any ♀ | |
| 777 | 17 | 22 | 51 | 67 | 0 | 0 | 3 | 5 | 165 |
| 803 | 19 | 34 | 57 | 47 | 0 | 2 | 4 | 4 | 167 |
| 804 | 28 | 20 | 33 | 37 | 1 | 1 | 4 | 4 | 128 |
| 806 | 44 | 40 | 60 | 41 | 1 | 1 | 5 | 2 | 194 |
| 807 | 18 | 19 | 31 | 27 | 2 | 1 | 7 | 3 | 108 |
| 808 | 37 | 19 | 31 | 44 | 1 | 2 | 7 | 4 | 145 |
| 809 | 11 | 18 | 35 | 32 | 1 | 1 | 3 | 2 | 103 |
| 857 | 8 | 22 | 13 | 31 | 0 | 0 | 3 | 3 | 80 |
| 858 | 8 | 4 | 9 | 10 | 0 | 0 | 1 | 2 | 34 |
| 859 | 8 | 21 | 28 | 35 | 0 | 1 | 2 | 6 | 101 |
| 860 | 18 | 29 | 24 | 43 | 1 | 0 | 1 | 3 | 119 |
| 861 | 29 | 38 | 54 | 44 | 1 | 2 | 4 | 7 | 179 |
| 863 | 23 | 34 | 34 | 35 | 0 | 0 | 7 | 3 | 136 |
| 864 | 20 | 9 | 20 | 23 | 0 | 1 | 0 | 5 | 78 |
| 865 | 4 | 2 | 8 | 0 | 1 | 0 | 0 | 0 | 15 |
| 866 | 24 | 29 | 35 | 37 | 0 | 0 | 4 | 3 | 132 |
| 867 | 30 | 24 | 12 | 30 | 1 | 1 | 1 | 3 | 102 |
| 868 | 21 | 24 | 25 | 29 | 0 | 0 | 2 | 3 | 104 |
| 869 | 30 | 40 | 26 | 43 | 0 | 1 | 4 | 3 | 147 |
| 870 | 31 | 31 | 45 | 45 | 1 | 2 | 2 | 3 | 160 |
| 871 | 23 | 24 | 16 | 23 | 1 | 2 | 1 | 1 | 91 |
| 872 | 26 | 16 | 27 | 21 | 0 | 2 | 3 | 2 | 97 |
| 873 | 33 | 39 | 34 | 48 | 2 | 1 | 3 | 11 | 171 |
| 874 | 20 | 18 | 26 | 24 | 0 | 2 | 5 | 1 | 96 |
| 875 | 29 | 25 | 27 | 18 | 0 | 1 | 4 | 0 | 104 |
| 876 | 10 | 9 | 14 | 12 | 0 | 0 | 0 | 2 | 47 |
| 877 | 18 | 21 | 25 | 18 | 0 | 0 | 0 | 4 | 86 |
| Totals | 587 | 631 | 800 | 864 | 14 | 24 | 80 | 89 | 3089 |

Total flies 3089; total crossovers 207; crossover percentage 6.7.

TABLE 2

*Linkage of Red and Orange. Red Orange* $\frac{ro}{ro}$ *males* $\times$ $\frac{RO}{ro}$ *females.*

| CULTURE NUMBER | NON CROSSOVERS | | | | CROSSOVERS | | | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| | Mahogany ♂ | Mahogany ♀ | Red Orange ♂ | Red Orange ♀ | Red ♂ | Red ♀ | Orange ♂ | Orange ♀ | |
| 936 | 54 | 55 | 26 | 25 | 2 | 2 | 2 | 4 | 170 |
| 937 | 31 | 47 | 14 | 30 | 4 | 2 | 2 | 4 | 134 |
| 938 | 26 | 43 | 39 | 32 | 1 | 0 | 6 | 3 | 150 |
| 939 | 37 | 39 | 25 | 26 | 2 | 0 | 2 | 2 | 133 |
| 940 | 36 | 35 | 42 | 29 | 2 | 2 | 4 | 3 | 153 |
| 941 | 33 | 45 | 33 | 30 | 1 | 4 | 2 | 4 | 152 |
| 942 | 48 | 38 | 21 | 23 | 4 | 2 | 0 | 0 | 136 |
| Totals | 265 | 302 | 200 | 195 | 16 | 12 | 18 | 20 | 1028 |

Total flies 1028; total crossovers 66; crossover percentage 6.42.

Two crosses of vermilion facet females to vermilion facet males and seven crosses of vermilion orange females to vermilion facet males were made. Although subcultured twice none of these produced any offspring. Facet females were then selected and crossed to vermilion orange males. These crosses produced wild type offspring, showing that facet was recessive and not sex-linked. In the $F_2$ generation some facet orange flies appeared indicating that facet was not linked to red, orange and triangle. Fully thirty facet males have been tested and all have been entirely sterile. The females, however, are of normal fertility and the viability of facet is excellent. The stock is maintained by selecting in each generation homozygous facet females and crossing to their heterozygous facet brothers.

### EXTRA-SCUTELLARS (*e*), A RECESSIVE BRISTLE CHARACTER

On March 14th, 1927, in culture 993, which was the $F_2$ generation of a cross of the sex-linked vermilion male to a female from a stock received from Doctor A. H. STURTEVANT from Kansas City, Kansas, two vermilion females and one vermilion male were found having small extra-scutellar bristles. These were mated and a large percentage of the flies from this cross had extra-scutellars. Subsequent tests have shown the character to be an autosomal recessive and not linked either to red, orange, and triangle or to facet. The character expression varies from one to three extra-scutellars. The bristles are smaller than the other scutellars and the most frequent position is just back of the anterior scutellars, or in the midline at the posterior edge of the scutellum. Where three bristles

are present one is found in the midline and one on either side. Generally in homozygous cultures some of the flies fail to show the character, and as in the case of triangle the percentage of such normal overlaps is quite variable. For this reason the mutant is of little value other than at present it represents a new linkage group in *D. hydei*.

## DISCUSSION AND SUMMARY

METZ and MOSES (1923) have described the chromosome groups of many species of drosophila. According to their figure that of *D. hydei* consists of six pairs of chromosomes of which one pair is very small. The mutations reported in this and other papers fall into four linkage groups. Those in the X chromosome with their approximate loci are Notched (0.0) white (3.8), vermilion (15.5), and bobbed (63). In the first autosome lie red (0.0) orange (6.6), and triangle (locus not accurately determined). Facet lies in the second autosome and extra-scutellars in the third autosome. It is not unlikely that continued investigation of the species will result in the finding of a fifth linkage group, and possibly of a sixth. The finding of four sex-linked mutants similar to sex-linked mutants in *D. melanogaster* and of three linked autosomal mutants, red, orange, and triangle similar to the three third chromosome mutants of *D. melanogaster*, scarlet, peach, and triangle is additional evidence indicating that there is some correlation in the configuration of the chromosome maps of various species of Drosophila.

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# INHERITANCE STUDIES IN SOYBEANS. III. SEED-COAT COLOR AND SUMMARY OF ALL OTHER MENDELIAN CHARACTERS THUS FAR REPORTED[1]

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## INTRODUCTION

In 1923 the writer began a study of the inheritance of seed-coat colors in the soybean (*Soja max*). From a practical standpoint, the problem is of great interest because seed-coat colors are very important in the selection of pure strains and in the detection of mixtures in commercial varieties. This led to a rather intensive study of the factors concerned in mottling, the results of which are being published in another paper.

The results of previous workers indicated that two varieties may look alike but may behave very differently in crosses. This made it advisable to secure numerous crosses among a large number of varieties in order that the genetic constitutions of different types could be compared. Al-

[1] From the Department of Genetics Paper No. 74 and the Department of Agronomy, WISCONSIN AGRICULTURAL EXPERIMENT STATION. Published with the approval of the Director of the Station.

though the original plans have by no means been realized, over 300 hybrids, representing 35 different combinations, have been produced.

Some of the parental plants used for hybridization were from well known varieties, but others were from selections made by the Agronomy Department of the WISCONSIN AGRICULTURAL EXPERIMENT STATION, and their origin is uncertain. It has been necessary to assign arbitrary numbers to these selections which have not been identified as belonging to any particular variety.

With but few exceptions all factors studied in soybeans have something to do with the color of the seed coat, either directly or through linkage. It seems logical, therefore, to center the discussion on seed-coat colors although a complete list is given of all the genetic factors that have been reported.

In carrying out the problem the writer has been greatly aided by counsel from Professor B. D. LEITH of the Agronomy Department and Professors L. J. COLE and R. A. BRINK of the Genetics Department of the UNIVERSITY OF WISCONSIN. It is a pleasure to acknowledge all the advice and constructive criticisms which have been received.

## SEED-COAT COLOR TYPES

NAGAI (1921) and PIPER and MORSE (1923) have given classifications of seed-coat color types for soybeans, but for the purpose of interpreting Mendelian characters the following classification has been found most useful.

I. Self-color type
   1. Black
   2. Brown (may be of different intensities according to the variety and somewhat according to conditions at the time of maturity)
   3. Imperfect black } (Associated with gray pubescence)
   4. Buff }

II. Bicolor type
   1. Black mottling pattern on brown background

III. Eyebrow pattern with green or yellow background
   1. Black
   2. Brown
   3. Imperfect black } (Associated with gray pubescence)
   4. Buff }

IV. Green or yellow seed coat with dark hilum. (Mottling is developed over the remaining portion of the seed coat according to environmental conditions.)

1. Hilum black
2. Hilum brown
3. Hilum imperfect black } (Associated with gray pubescence)
4. Hilum buff }

V. Green or yellow seed coat with light hilum. (Mottling is produced according to environmental conditions but pigments are always potentially present.)
1. Mottled with black
2. Mottled with brown
3. Mottled with imperfect black } (Associated with gray pubescence)
4. Mottled with buff }

### PIGMENTS RESPONSIBLE FOR SEED-COAT COLORS

What has been designated as black pigment in the seed coat of the soybean is really a very intense purple and belongs to the general class of anthocyanins. The brown or phlobaphene-like pigments are undoubtedly closely related to quercetin but their exact chemical composition is not known. In the imperfect blacks there is a mixture of the two pigments and even in the self-blacks a considerable amount of brown pigment is masked by the black.

In addition to the pigments mentioned above, which are glucosides and have many properties in common, there is another blackish-brown pigment which behaves very differently. In all probability it belongs to the general class known as "oxidation pigments" because of their connection with the process of oxidation. This is the pigment responsible for the common blackish-brown pod color in black-podded varieties but this same pigment may also find its way into the parenchymatous cells of the seed-coat. Frequently it produces such a smudgy appearance that a classification of a plant in regard to the other pigments is rather uncertain. Unlike the anthocyanins and phlobaphene-like pigments, the production of the oxidation pigment may be greatly increased by injury to the pod and testa.

Another colored condition has been described by MATSUMOTO and TOMOYASU (1925) which they have called "purple speck." The pigment responsible for this abnormal coloration, according to the descriptions, has similar properties to anthocyanins but a fungus (*Cercosporina Kikuchii*) was found to be responsible for its occurrence.

The black and brown pigments, with which the present article is chiefly concerned, are independent of the green and yellow plastid pigments. In self-browns or mottled types not all of the plastid color is concealed,

so it is usually possible to detect the presence of green when it is present but in self-blacks plastid colors are entirely masked.

## THE VARIABILITY OF SEED-COAT COLORS

Except in self-colored types there is a great deal of variability in the amount of pigment produced in the seed coat. This has been regarded as objectionable by practical growers, so the subject of mottling has received considerable attention in all the important soybean-growing sections of the United States. Much effort has been spent in selecting for non-mottled strains but all varieties which have been tested in cooperation with the Agronomy Department of the WISCONSIN AGRICULTURAL EXPERIMENT STATION have produced mottling in some degree. Some selections have proved to be much more badly affected than others but mottling seems to be a general phenomenon with all yellow- and green-seeded varieties. Details of this work are being published in another paper.

WOODWORTH and COLE (1924) have given evidence to show that environmental factors may be important in bringing out mottling pigments and their conclusions have been well confirmed during the progress of this study. Varieties which failed to develop any signs of mottling in the greenhouse during winter months were badly mottled in the field.

A rich loam soil has consistently produced much more mottling than a light sandy soil, but the distance of spacing plants has also been found to be very important. Plants grown three feet apart have produced more mottled seed than plants grown closely together, but on certain types of soil the distance of spacing has not been a determining factor. These results are confirmed by HOLLOWELL'S (1924) findings at Iowa.

Although it seems probable that there may be many factors which affect mottling, perhaps the most important is the maturation of the seed. An extreme amount of mottling was found to be associated with plants which remained green after the normal time for the seeds to mature.

Environmental factors have no apparent influence in the case of self-colored varieties, because no difficulty is experienced in getting these varieties to breed true; but there are noticeable differences in intensity of color due to environment. The imperfect-black type is extremely variable in its appearance. Sometimes seeds are nearly black and in other cases some experience is required to distinguish this type from light brown. NAGAI (1924) gives evidence to show that this variability is not independent of inheritance but the present writer has no data on the relative importance of environment and heredity on this particular coloration.

The self-browns and buffs are also variable in regard to the intensity of color and conditions at maturity seem important in this connection.

For a genetic study of seed-coat colors it is usually desirable to have the plants growing in an environment that will produce the maximum amount of pigment. This together with an intimate knowledge of the variability that might be expected due to environment is necessary in order to classify intelligently certain $F_2$ progenies.

## RESULTS OF PREVIOUS INVESTIGATIONS

PIPER and MORSE (1923) found black seed coat dominant to brown. In the particular example cited a single factor was responsible for the difference. NAGAI (1921) also found black to be dominant to brown but he distinguished between different types of blacks and browns. He demonstrated the presence of a factor *R* which produced anthocyanin pigment and a factor *C* which increased the intensity of pigmentation. According to NAGAI'S hypothesis, with *CR* present the seed coat is black; with *cR* it is an imperfect black; with *Cr* it is brown; and with *cr* it is a very light brown or "buff."

In addition to *C* and *R*, NAGAI assumed that another factor *O* must be present in order to explain the inheritance of certain browns. A rich reddish brown was recessive to the common dull brown. Therefore, with *CO* present the pigment was a dull brown, and with *Co*, reddish brown; but the relation between the two factors, *C* and *O*, was not entirely clear.

WOODWORTH (1921) observed that two factors, *B* and *H*, were necessary for the production of black pigment in the hilum, but *H* was found to be completely linked to *T*, the factor for tawny pubescence. Plants homozygous for *ht* always produced gray pubescence and a seed coat with a brown hilum. In Part II of this series another factor, *E*, was added to this linkage group. This factor *E* is a partially dominant factor for early maturity but since the expression was not always clear-cut it was only through the linkage relation that its effect could be attributed to a single factor. There was evidently a small percentage of crossing over but this could not be accurately measured.

NAGAI and SAITO (1923) found self-blacks or self-browns recessive to yellow or green and they have described three restriction factors. According to these writers, the factor *K* inhibits the production of pigments forming a "patched" or "eyebrow" pattern; *I* inhibits nearly completely the formation of chromogenic substance; and *H* inhibits it entirely. The recessives of these factors have no effect.

NAGAI and SAITO also found a single factor *M* responsible for the production of a black mottling pattern upon a brown background. They assumed that *M* is linked to the factor *P* which inhibits the formation of pubescence, giving a crossover value of approximately 18 percent. In Part II of this series the writer also reports a linkage between *P* and $R_1$, a factor for anthocyanin pigment. This linkage appears to be nearly complete with only occasional crossovers. Mention is also made of the factor $p_2$ for glabrousness reported by STEWART and WENTZ (1926) which was recessive to $P_2$, a factor for pubescence. Thus we have a character (glabrousness) which is dominant in one case and recessive in another case; apparently due to different factors.

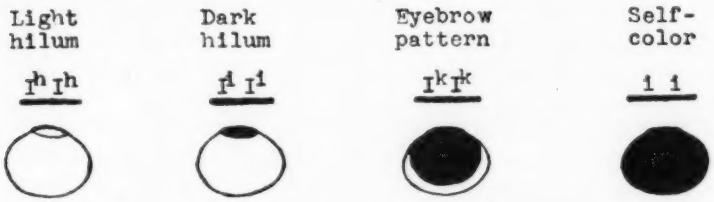
TAKAHASHI and FUKUYAMA (1919) have given results obtained in $F_2$ from five different crosses but no attempt was made to give an interpretation, due to the small amount of data available. Their results at least indicate that varieties are green or yellow because of the presence of factors that inhibit pigment formation. More blacks than browns were secured in the $F_2$'s but the data do not warrant conclusions in regard to the factorial relationships.

NAGAI (1921) found that black and brown pigments are formed regardless of whether the plastid pigments in the seed coat are green or yellow. Green, however, was dominant over yellow. WOODWORTH (1921) obtained identical results but found that *V*, the factor used for green seed coat, was linked with *D*, a factor for the production of yellow cotyledons, giving about 13 percent crossing over; but *V* was assumed to be independent of another factor *I* which is a duplicate of *D* and also produced yellow cotyledons. These findings have been well confirmed by the writer and the results are briefly presented in Part I of this series.

TERAO (1918) found a single factor responsible for green seed coat color when the maternal parent was yellow, but when the maternal parent was green there was no segregation, the $F_1$'s and $F_2$'s being the same color as the female parent. PIPER and MORSE (1923) confirm TERAO's findings, and both writers have postulated that two types of green are responsible for the odd results. Although the inheritance of the green seed coat was independent of the paternal parent yet two types of greens were distinguished in the $F_2$ progenies of green seed-coat ♀ × yellow seed-coat ♂. One type was more intense than the other and when counts were made it was found that the results were approximately in accord with a ratio of 3 dark green to 1 light green. One type of green was therefore inherited according to Mendelian expectation while the other type was a clear case of maternal inheritance.

## ALLELOMORPHIC SERIES FOR THE RESTRICTION OF PIGMENT IN THE SEED COAT

NAGAI and SAITO (1923) postulated that the three restriction factors which they described as *H*, *I*, and *K*, were allelomorphic. This assumption has been well confirmed in the present study, so there seems to be no good reason for not making this clear in the terminology. Four allelomorphs may, therefore, be described according to their inhibiting effect upon the production of pigment in the seed coat. The following figure illustrates the action of the factors which have been found in this allelomorphic series.




As the above figure suggests $I^h$, under the proper environmental conditions, inhibits all pigment formation in the seed coat; $I^i$ inhibits pigments to the hilum; $I^k$ inhibits the formation of pigment in such a way as to form the "eyebrow" or "patched" pattern; and *i*, the recessive allelomorph, has no effect.

Although it is necessary to define these restriction factors rather definitely, it should be clearly kept in mind that environmental conditions unfavorable for pigment formation are necessary in order for these restriction factors to fully express themselves. Varieties with the genetic constitution *i i* come under Class I or II of the "Seed-coat Color Types." In these two classes there is no restriction of the pigment and environment has practically no effect but in classes III, IV, and V, which $I^kI^k$, $I^iI^i$, and $I^hI^h$ represent respectively, environmental conditions are very important in addition to the hereditary factors. Thus, varieties which come in Class V are of constitution $I^hI^h$, but may develop considerable mottling. From individual seeds it would frequently be impossible to distinguish the three types because of the variation due to environment but when all the seeds from a plant are available for classification there is usually no difficulty. It should be mentioned, however, that these restriction factors are only partially dominant. A cross between $I^hI^h$ and *i i* invariably

produces an $F_1$ that is mottled more than plants homozygous for $I^h$ and in such cases it is sometimes difficult to distinguish the color types.

Unfortunately the writer was not successful in obtaining a cross between varieties of constitution $i\ i$ and $I^kI^k$ but NAGAI and SAITO (1923) have already described this relationship. All other combinations, however, have been made and the results from these crosses constitute a critical test for the theory that $I^h$, $I^i$, $I^k$, and $i$ are allelomorphic. The best evidence for this relationship rests upon the results of the three combinations of crosses between Mandarins ($I^hI^h$), Soysotas ($i\ i$) and Manchus ($I^iI^i$). The parental plants of the Mandarin and Soysota varieties were from strains selected which were known to be homozygous, and the Manchu selection (No. 59), which will be referred to here, was also known to be homozygous.

The distribution of dominant and recessive types in all $F_2$ progenies closely approached a 3:1 ratio and the crucial proof for the allelomorphic series rests upon this simple but consistent behavior. The following tabulation indicates this relationship.

| | *Dominant* | *Recessive* | *Deviation* |
|---|---|---|---|
| Mandarin ($I^hI^h$)×Soyosota ($i\ i$) | 15 | 5 | 0.0±1.3 |
| Manchu ($I^iI^i$)×Soyosota ($i\ i$) | 250 | 92 | 6.5±5.4 |
| Mandarin ($I^hI^h$)×Manchu ($I^iI^i$)[1] | 223 | 75 | 2.0±5.0 |

Any explanation other than that of an allelomorphic series is obviously precluded because this is the only way in which a 3:1 ratio can be secured for each of the combinations. If the Mandarin variety were of constitution *HHii* and if the Manchu were *hhII* (*H* being synonymous with $I^h$ and *I* synonymous with $I^i$) the crosses with the Soysota variety could be explained because it may have the constitution *hhii*. Such a theory is shattered when the Mandarin is crossed with the Manchu because with two factors involved there would be a dihybrid ratio and 1/16 of the plants in $F_2$ would be self-colored, but none were observed. The probability of this happening when 412 plants are grown would be $(15/16)^{412}$ or the odds would be over $10^{11}$:1, and results from other similar crosses are also against a two factor difference.

The following crosses were also obtained in which the factor $I^k$ is involved. In the cross Black Eyebrow ($I^kI^k$)×Glabrous No. 5 ($I^iI^i$), 186 plants produced seed with the pigment restricted to the hilum and 65 produced seed having the eyebrow pattern. Based on a 3:1 ratio the

[1] There were 412 $F_2$ plants in this cross but only tawny-pubescent plants were classified for reasons which will follow in the text.

deviation is $2.3 \pm 4.6$. If $I^k$ were not an allelomorph of $I^i$ then the Glabrous No. 5 parent would necessarily have had the two dominant factors $I^i$ and $I^k$. Such an assumption is not in accord with the breeding facts from crosses between varieties with pigment restricted and self-color because in every case a single factor difference explains the results.

In order to furnish further proof for the allelomorphic series it would have been desirable to have backcrossed an $F_1$ plant of constitution $I^kI^i$ or $I^kI^k$ to the self-colored $i\,i$ variety. Such crosses should bring out only the two dominant types in the next generation, and the proof for the allelomorphic series would be complete. Due to the difficulty in crossing soybeans, however, this task would be difficult to carry out and the indirect evidence which is already available is practically conclusive that $I^k$, $I^i$, $I^k$, and $i$ occupy identical positions on the chromosomes.

*Restriction of pigment to the hilum*

It is impossible to assert definitely that a factor identical with NAGAI and SAITO's factor $I$ has been found, but at least it is convenient to use such a designation. This factor $I^i$, as it is being defined here, is responsible for restricting pigments to the hilum.

The Manchu variety has a yellow seed coat with a black hilum and results have shown that the reason for the seed coat being yellow instead of black is due to the presence of $I^i$, the dominant restriction factor. Environmental factors produce great variations in the amount of mottling developed; but the hilum remains colored in spite of the fact that environmental conditions may be very unfavorable for the development of mottling.

With no restriction factors other than $I^i$ present the hilum is always black or brown, but the intensity of the color may depend on other factors. In tawny-pubescent varieties the hilum color is prominent but in gray-pubescent varieties the color may be very faint. Varieties differ also in regard to size of hilum and this likewise seems to determine the prominence of the color. Of course, we can not be absolutely sure from the data now available that the factor responsible for restricting pigments to the hilum has been the same one in every case, but neither have we proof for the contrary. It seems logical, therefore, to assume that $I^i$ is responsible for restricting pigments to the hilum.

Three different strains of Manchus were crossed with self-browns but each of these combinations gave very similar results. Table 4 gives the detailed behavior of these crosses but the combined results obtained in $F_2$ are the following:

| *Phenotypes* | *Black hilum* $R_1I^i$ | *Brown hilum* $r_1I^i$ | *Self-black* $R_1i$ | *Self-brown* $r_1i$ |
|---|---|---|---|---|
| Observed | 584 | 169 | 171 | 71 |
| *Calculated* | *559.7* | *186.6* | *186.6* | *62.2* |
| Difference | +24.3 | −17.6 | −15.6 | +8.8 |

The value of $\chi^2$ is 5.26 assuming independence between $I^i$, the restriction factor, and $R_1$ the factor for blaek pigment. This gives P a value of 0.16.

Another cross already mentioned was made between the Black Eyebrow variety ($I^kI^k$) and a glabrous Japanese variety ($I^iI^i$) (No. J5) with a brown hilum. The Black Eyebrow variety is characterized by the eyebrow pattern that has been described so we assume that the restriction factor $I^k$ is present. The factor $I^i$ has the same action with the eyebrow pattern as with the self-color, inhibiting the development of pigment outside of the hilum in both cases. The results are as follows:

| *Phenotypes* | *Black hilum* $R_1I^i$ | *Brown hilum* $r_1I^i$ | *Black eyebrow* $R_1I^k$ | *Brown eyebrow* $r_1I^k$ |
|---|---|---|---|---|
| Observed | 136 | 50 | 47 | 18 |
| *Calculated* | *141.2* | *47.1* | *47.1* | *15.7* |
| Difference | −5.2 | +2.9 | −0.1 | +2.3 |

It may be safely assumed that $I^i$ and $R_1$ were independent because of the small deviations from the theoretical 9:3:3:1 ratio and the value for $\chi^2$ is 0.329 and P = 0.9+.

### *Restriction of pigment over the entire seed coat*

NAGAI and SAITO (1923) have described a factor $H$ which completely inhibits the development of brown and black pigments. They neglect to state that mottling is possible when $H$ is present but there are many reasons to believe that such is the case. The writer's experience has been that the expression of this factor, $I^h$ according to the new usage, may be greatly influenced by environmental conditions. All pigment may be entirely restricted or there may be considerable mottling. The Mandarin, Aksarben, and Ito San varieties have such restriction factors and these three varieties have been crossed rather extensively.

When a self-color (brown or black) is crossed with a variety possessing the restriction factor $I^h$, the $F_1$ has a strong tendency to be intermediate. Even in the greenhouse, where conditions extremely unfavorable for the production of mottling were provided, heterozygous plants produced seeds slightly speckled or blotched with pigment. This condition was not apparent, however, when the cross was made with the Manchu variety

which possessed the factor $I^i$ for the restriction of pigment to the hilum. It is to be assumed, therefore, that with both $I^i$ and $I^h$ present the tendency for a restriction of pigment is stronger than if only one $I^h$ is present.

In order to have a convenient method of referring to pigment patterns the designations used in the classification of color types have been adhered to. "Dark hilum," therefore, means that the hilum is colored as in the Manchu variety but the pigment may be black or brown. The factor $I^i$ is responsible for inhibiting the pigment to the hilum in this manner. "Light hilum" means that $I^h$ is present and no pigment is formed except that which may be due to mottling.

No difficulty was experienced in classifying tawny-pubescent plants according to whether their seeds had dark or light hilums, but with gray-pubescent plants the distinction has not always been clear because of the faint development of pigment. In some way the presence of $T$ seems to intensify seed-coat pigments and this helps materially in classifying plants for the presence or absence of $I^h$.

Four different combinations between self-browns and yellows have been made. The detailed results are given in Table 5 but the combined results are the following:

| *Phenotypes* | *Tawny mottled* $TI^h$ | *Tawny self-brown* $Ti$ | *Gray mottled* $tI^h$ | *Gray, Buff* $ti$ |
|---|---|---|---|---|
| Observed | 137 | 48 | 38 | 22 |
| *Calculated* | *137.8* | *45.9* | *45.9* | *15.3* |
| Difference | −0.8 | +2.1 | −7.9 | +6.7 |

A comparison with calculated results, on the basis of independence, gives deviations that may be accounted for as being due to chance; the value of $\chi^2$ is 4.39 and $P = 0.23$.

Six combinations between black-seeded varieties and varieties possessing the factor $I^h$ were also secured (table 1). The combined $F_2$ data from these crosses are as follows:

| *Mottled or colorless* | *Self-colored* | *Dev.* | *P. E.* | $\frac{Dev.}{P.E.}$ |
|---|---|---|---|---|
| 518 | 186 | 10.0 | 7.75 | 1.3 |

These data again show a fairly good agreement between observed figures and the theoretical 3:1 ratio.

A cross, Black Eyebrow ($I^hI^h$) × Ito San ($I^hI^h$), was also made which gave an actual ratio of 18 with pigment restricted to 4 with the eyebrow pattern. These data are almost in perfect agreement with theoretical figures, the deviation being $1.5 \pm 1.4$.

A cross between a Manchu No. 1 ($I^iI^i$) and an unidentified yellow variety ($I^hI^h$), designated as No. 12 in the records, gave the following results in $F_2$.

| | LIGHT HILUM | | DARK HILUM | |
|---|---|---|---|---|
| | Black | Brown | Black | Brown |
| Observed | 120 | 36 | 51 | 14 |
| *Calculated* | *124.4* | *41.4* | *41.4* | *13.8* |
| Difference | −4.4 | −5.4 | +9.6 | +0.2 |

Comparing the observed data with the calculated, assuming that $I^h$ is independent of $R_1$, $\chi^2=3.10$ and $P=0.38$. The behavior of $F_3$ progenies also corroborates the results obtained in $F_2$ and these data are given in table 6.

Six combinations between light hilum and gray pubescence ($t\ I^h$)×dark hilum and tawny pubescence ($T\ I^i$) were secured. From a general observation of these $F_2$'s one would naturally assume that $I^i$ is completely linked with $T$, because no plants with gray pubescence bore seed with the typical dark hilum. If complete linkage is assumed we have the following results compared with the theoretical 2:1:1 ratio.

| | *Tawny, Light hilum* | *Tawny, Dark hilum* | *Gray, Light hilum* | *Gray, Dark hilum* |
|---|---|---|---|---|
| Observed | 709 | 235 | 299 | |
| *Calculated* | *621.5* | *310.7* | *310.7* | |
| Difference | +87.5 | −75.7 | −11.7 | |

With this comparison $\chi^2=31.20$ and $P=0.000001$, so it is unlikely that the deviations could be due to chance. Since $T$, the factor for tawny pubescence, has a marked effect on both the quantity and quality of pigments, one may assume that some gray-pubescent plants really had dark hilums but could not be easily distinguished. Recalculating with this assumption we get the following comparison with the 9:3:4 ratio.

| | *Tawny, Light hilum* | *Tawny, Dark hilum* | *Gray, (Hilum color not distinguished)* |
|---|---|---|---|
| Observed | 709 | 235 | 299 |
| *Calculated* | *699.2* | *233.1* | *310.7* |
| Difference | +9.8 | +1.9 | −11.7 |

In this case $\chi^2=0.59$ and $P=0.9$, showing a very good agreement between observed and expected results.

A very similar cross between the dark and light hilums has been described by WOODWORTH (1921) but he was unable to classify his $F_2$ plants, because of the irregular pigment which was probably due to environmental conditions.

*Restriction of pigment to form an eyebrow pattern*

Although the three restriction factors $I^k$, $I^i$, $I^k$ seem well established, there are some facts which indicate a larger series of allelomorphs. It has already been mentioned that some strains of Manchus have been selected which mottle much more badly than others, but selection within any one strain has not been successful. In other words these strains must have been homozygous to begin with, and if mutations occur they have not been with sufficient frequency to be selected. In progeny tests with selections from the Black Eyebrow variety, however, heritable variations from the normal eyebrow pattern have been observed, indicating the presence of another factor which restricts the pigment less than $I^k$, but it is possible that modifying factors were involved.

One selection (No. 84) bred true for the normal eyebrow pattern for four generations and the uniformity was very remarkable. Another selection (No. 82) bred true for a very dark pattern, and in this progeny plants with half of their seeds completely black were not uncommon, but no plant produced 100 percent black seeds. A third progeny (No. 83) was apparently heterozygous for factors affecting the eyebrow pattern because selections were made which bred true for a normal eyebrow pattern; others bred true for a very dark pattern; and still other selections continued to produce some plants with normal patterns and others with dark patterns.

RELATION BETWEEN BLACK AND BROWN PIGMENT

It has been pointed out that self-black is dominant to self-brown in the seed coat and that ratios of 3 blacks to 1 brown are secured in $F_2$. The first cross made by the writer was of this type and the following results were obtained.

| *Black* | *Brown* | *Dev.* | *P. E.* |
|---|---|---|---|
| 72 | 28 | 3 | 2.92 |

In this cross (Black, No. 3×Brown, No. 30)[1] the $F_1$ plant bore seed fully as black as the maternal parent, and in the $F_2$'s there was no visible

[1] This progeny also segregated for an unusual type of hilum peculiar to the Soysota variety, the paternal parent. Ordinarily there is a clean separation between the hilum and the funiculus in soybeans, but in this abnormal type the Malpighian cells are arranged in such a way that the funiculus is torn, leaving a rough surface that is very characteristic. This condition is recessive

difference between the intensity of blacks, that is, black was fully dominant to brown.

In 1923 a natural hybrid (Selection No. 60) was accidentally found that segregated according to a ratio of 3 blacks to 1 brown in the progeny that was grown, but all heterozygous plants bore seed slightly speckled with brown. The results were as follows:

| | *Black* | *Dilute black* | *Brown* |
|---|---|---|---|
| Observed | 7 | 14 | 8 |
| *Calculated* | *7.25* | *14.50* | *7.25* |

It so happened that the observed distribution was exactly in accord with the calculated except for an extra brown. Results in $F_3$ proved that all of the 14 plants with dilute black were actually heterozygous. The peculiar behavior of the black pigment is noticeable only in the heterozygous condition. In homozygous plants the black is apparently just as intense as that produced by any other variety. It is, therefore, a simple case of incomplete dominance. This kind of partial dominance is well known, and 1:2:1 ratios are perhaps as much to be expected as 3:1 ratios. BLAKESLEE and AVERY (1917) have reported a case of incomplete dominance of mottling in the Adzuki bean (said to be *Phaseolus Mungo*) that is very similar to this lack of dominance in the seed-coat color of the soybean.

The difference between the two kinds of blacks appeared interesting, so crosses were made using the hybrid $F_2$ plants for parental material. Crosses with types possessing the dominant inhibiting factor $I^h$ were secured. Actually this was equivalent to back-crossing because in some cases heterozygous plants were used as parents. Therefore, combinations with $r_1$ were secured as well as with $R_1$.

Two combinations with $R_1$ are given in table 1, Mandarin and Aksarben varieties being the other parents. Without considering the details of the table at this time it may be of interest to state that out of 17 self-blacks grown in 1925 from these crosses 10 were dilute black and were undoubtedly heterozygous, as was previously found to be the case.

## FLOWER AND PUBESCENCE COLORS IN RELATION TO SEED-COAT PIGMENTS

In table 1 six crosses are compared between blacks with a factor present for the production of anthocyanin pigment and yellows with $I^h$ present for the restriction of all pigment. It has already been pointed out that

to the normal hilum and in all crosses it has proved to be independent of all other perceptible characters. Tables 9 and 10 give the evidence for this statement, the factor for normal hilum being designated as *N* and that of the Soysota variety as *n*.

black × brown has usually given 3:1 ratios, black being dominant. WOODWORTH'S (1921) example, where complementary factors were involved giving a 9:7 ratio, is the only exception reported.

In the crosses reported in table 1 three strains of blacks (No. 3, No. 29,[1] and No. 60) and four strains of yellows (Mandarins, Ito Sans, Aksarbens, and No. C3) were used. No. C3 is an unidentified Chinese variety, and it resembles the Mandarin variety very much but the flowers are white. It may also be said that this is the only white-flowered variety used in the crosses referred to in table 1. The other six parents had the ordinary purple flowers which are dominant to white.

Black No. 60 was a selection from the natural hybrid that has been described, the peculiar thing about this selection being the incomplete dominance of the black seed-coat color in the heterozygous condition.

Table 1 affords an opportunity for a study of pubescence colors in relation to seed-coat colors. Ito Sans have tawny pubescence while the other three yellow-seeded varieties have gray pubescence. When Ito Sans were crossed with blacks a 9:3:3:1 ratio resulted in $F_2$ and the results can be explained due to the presence of $R_1$ in the blacks and $I^h$ in the Ito Sans, but these results are quite different from those obtained when gray-pubescent varieties were used as parental stock. When Mandarins and the Aksarbens were crossed with blacks a 27:9:9:3:9:3:3:1 ratio was found to explain the results. It will be noted that rather high values for $\chi^2$ are obtained for these crosses in table 1 but the numbers are rather small and the fact that all classes are represented may be of some significance. Combining progenies, 133, 139, 172, 175, 171, and 164 $\chi^2 = 14.52$ and $P = 0.04$. Both the black and the brown pigments were influenced in some way by the presence of *T*. On plants with gray pubescence, of constitution *tt*, there was an incomplete development of pigment. The black and brown correspond with what NAGAI (1921) has described as "imperfect black" and "buff," respectively. It is therefore convenient to continue the use of these terms.

NAGAI does not state that imperfect black was associated with gray pubescence but this has been the case in progenies 133, 172, and 164 (table 1). The logical assumption, therefore, is to consider *T* identical with NAGAI'S factor *C*. If these factors are not identical they are at least completely linked, because tawny pubescence has always been associated with a more intense development of both the anthocyanin and phlobaphene-like pigments. These are the exact properties which NAGAI has attributed to his factor *C*.

[1] Selection from Wisconsin Black variety. Nos. 3 and 60 could not be identified as to variety.

TABLE 1

*$F_2$ progenies of black×yellow seed coats.*

| | PROGENY NUMBERS | TAWNY PUBESCENCE | | | | GRAY PUBESCENCE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Black | | Brown | | Imperfect black | | Buff | | |
| | | Mottled | Self-color | Mottled | Self-color | Mottled | Self-color | Mottled | Self-color | |
| Black (No. 3) ×Ito San | 150 | 13 | 5 | 4 | 0 | | | | | |
| *Calculated* | | *13* | *4* | *4* | *1* | | | | | $\chi^2 = 1.25$ |
| Difference | | | +1 | | −1 | | | | | $P = 0.74$ |
| Black (No. 29) ×Ito San | 127 | 26 | 18 | 11 | 6 | | | | | $\chi^2 = 7.24$ |
| *Calculated* | | *34.4* | *11.4* | *11.4* | *3.8* | | | | | |
| Difference | | −8.3 | +6.6 | −0.4 | +2.2 | | | | | $P = 0.07$ |
| | 139[3] | 72 | 21 | 18 | 10 | 10 | 7 | 7 | 1 | |
| Black (No. 29) ×Mandarin | 133 | 31 | 7 | 6 | 7 | 8 | 3 | 2 | 2 | |
| | Total | 103 | 28 | 24 | 17 | 18 | 10 | 9 | 1 | $\chi^2 = 14.89$ |
| *Calculated* | | *88.6* | *29.5* | *29.5* | *9.8* | *29.5* | *9.8* | *9.8* | *3.3* | |
| Difference | | +14.4 | −1.5 | −5.5 | +7.2 | −11.5 | +0.2 | −0.8 | −2.3 | $P = 0.04$ |
| | 175 | 30 | 11 | 19 | 3 | 6 | 3 | 4 | 0 | |
| Black (No. 60) ×Mandarin | 172 | 26 | 6 | 10 | 2 | 5 | 3 | 7 | 3 | |
| | Total | 56 | 17 | 29 | 5 | 11 | 6 | 11 | 3 | |
| *Calculated* | | *58.2* | *19.4* | *19.4* | *6.5* | *19.4* | *6.5* | *6.5* | *2.2* | $\chi^2 = 12.56$ |
| Difference | | −2.2 | −2.4 | +9.6 | −1.5 | −8.4 | −0.5 | +4.5 | −0.8 | $P = 0.08$ |
| | 171[3] | 31 | 13 | 14 | 4 | 9 | 3 | 10 | 2 | |
| Black (No. 60) ×Aksarben (No. 53) | 164 | 25 | 11 | 10 | 0 | 8 | 2 | 4 | 2 | |
| | Total | 56 | 24 | 24 | 4 | 17 | 5 | 14 | 4 | |
| *Calculated* | | *62.4* | *20.8* | *20.8* | *6.9* | *20.8* | *6.9* | *6.9* | *2.3* | $\chi^2 = 12.64$ |
| Difference | | −6.4 | +3.2 | +3.2 | −2.9 | −3.8 | −1.9 | +7.1 | +1.7 | $P = 0.08$ |
| Black (No. 3) ×Yellow (No. C3) | 142 | 70 | 25 | | | 15[2] | 4[2] | 7[1] | 4[1] | $\chi^2 = 3.21$ |
| *Calculated* | | *70.3* | *23.4* | | | *17.6* | *5.9* | *5.9* | *2.0* | |
| Difference | | −0.3 | +1.6 | | | −2.6 | −1.9 | +1.1 | +2.0 | $P = 0.67$ |

[1] Plants also had white flowers.

[2] All plants had purple flowers.

[3] Progenies 139 and 171 are reciprocals of 133 and 164, respectively.

Progeny No. 142 [black No. 3 ($r_1R_2TW$)×yellow No. C3 ($r_1r_2tw$)] is of special interest because no browns appeared in the tawny-pubescent class, but in the gray-pubescent class there were both imperfect blacks and buffs. This gives a ratio of 15:1 for the presence versus absence of anthocyanin pigment. In the gray-pubescent class the imperfect blacks and buffs were very similar to seed similarly classified in the progenies 133, 172, and 164. $F_3$ progenies were also grown from $F_2$ plants of progeny 142. The original hypothesis was confirmed by these results. Although only 10 progenies from plants of phenotypic constitution ($T$ $R_2I^h$) were grown yet every possible genotypic arrangement was secured. Two segregated the same as the previous $F_2$ generation, five segregated for $I^h$ but not for $T$, and three segregated for $T$ but not for $I^h$. In the later progenies, $R_2$ was linked completely with $T$ and there were no signs of crossing over.

It is of special interest to note that the imperfect blacks in progeny 142 were also associated with purple flowers and that buffs were associated with white flowers. Therefore, it seems entirely probable that $W$, the factor for purple flowers (WOODWORTH 1923), may also be responsible for the development of anthocyanin pigment in the seed coat in this particular case. To obtain further evidence $F_3$ progenies of phenotypic constitution $T$ $R_2iw$ were grown in 1926 and two of these proved to be heterozygous for $T$. The linkage between $T$ and $R_2$ was again complete and all gray-pubescent plants produced buff seed-coat color. This evidence corroborates the previous breeding behavior in showing the necessity of the presence of $W$ before anthocyanin pigment can be produced in the seed coat of gray-pubescent plants.

WOODWORTH found that purple flower color was associated with purple hypocotyls but no effect on the seed coat has been reported; yet this condition is not surprising because in other plants the development of anthocyanin pigment has often been found to be associated with flower color. COMBES (1912) working with a species of Spiraea, found that coloration in the bark of twigs was easily brought about by decortication on species with colored flowers but this was not possible with species having white flowers.

As already stated, the crosses Black×Mandarin or Black×Aksarben can readily be explained by the three factors, $R_1$, $T$, and $I^h$. The restriction factor $I^h$ is independent of the other two factors, so there is no need of considering it at this time. The expression of the other two factors is as follows:

| | |
|---|---|
| $R_1T$ | Black, tawny |
| $R_1t$ | Imperfect black, gray |
| $r_1T$ | Brown, tawny |
| $r_1t$ | Buff, gray |

In the cross, Black (No. 3) × Yellow (No. C3), the situation is also simple but there is difficulty in explaining why the behavior is so different from other crosses. It cannot be said that $R_1$ is responsible for the production of anthocyanin in this case because of the complete linkage. Perhaps we can postulate that another factor $R_2$, not allelomorphic to $R_1$, is the factor for anthocyanin formation and that it is completely linked with $T$. This factor may be identical with Woodworth's factor $H$ because $H$ also produced black pigment and was completely linked with $T$. The factor $W$ has no apparent effect when $T$ is present but when the constitution was $tt$ it produced an imperfect black. The question then arises—why is it possible that $W$ can have this effect? If it had produced the same effect in Mandarins and Aksarbens the seed coats on these varieties should have been mottled with imperfect black instead of light brown because these are purple-flowered varieties. The genetic constitution of the yellow selection No. C3 must, therefore, differ from the genetic constitution of Mandarins in such a way that when $W$ is present a certain amount of brown pigment is converted into anthocyanin. Perhaps $r_2$, an allelomorph to $R_2$, makes this possible; and if this is the case a cross between the Mandarin (with $W$) and selection No. C3 (with $r_2$) should give imperfect blacks in $F_1$; i.e., the complementary factors $W$ and $r_2$ are necessary to explain the results.

On the basis of the above assumption the black selection No. 3 ($r_1R_2TW$) must have a factor $R_2$ which is different from the factor $R_1$ in No. 29 and No. 60, and selection No. C3 ($r_1r_2tw$) must also have an additional factor $r_2$ that is not present in Mandarins and Aksarbens. Then the results in progeny 142 can be explained as follows:

$r_1R_2TW$, $r_1R_2Tw$ ..... Black, tawny
$r_1r_2tW$ ..... Imperfect black, gray
$r_1r_2tw$ ..... Buff, gray

Since $r_2$ was completely linked with $t$, the single factor $T$ may be assumed to be responsible for the production of black seed coats as well as tawny pubescence, but results from other crosses show that $T$ does not always have this effect. When selection No. 3 ($r_1R_2T$) was crossed with the Ito San ($r_1r_2'T$) there were plants with brown seed coats and tawny pubescence in $F_2$ (table 1). Furthermore, $r_2'$ of the Ito San variety must be considered different from $r_2$ of selection No. C3 because $r_2$ was a complement of $W$ for the production of anthocyanin in the imperfect black seed-coats of progeny 142 (table 1), but $r_2'$ does not have this effect. From

these results it seems possible that an allelomorphic series may be situated at the $t$ locus. If this is true the following combinations have now been identified:

$R_2T$.....Tawny pubescence, black seed-coat (selection No. 3)
$r_2t$.....Gray pubescence, seed-coat imperfect black with $W$ but buff with $w$ (selection No. C3).
$r'_2T$.....Tawny pubescence, seed-coat brown with either $W$ or $w$ (Ito San variety)
$r'_2T$.....Gray pubescence, seed-coat brown with $W$ or $w$ (Mandarin and Aksarben varieties).

The identity of the factor $R_2$ for the production of anthocyanin pigment in the seed coat is well established, but it has been found in only one selection (No. 3). In selection Nos. 29 and 60 the factor ($R_1$) responsible for the production of anthocyanin pigment in the seed coat is clearly distinct from $R_2$, because, unlike the latter, it is not linked with $T$. There is also good reason for using the designation $R_1$ in selection No. C4 (table 3) but a number of other combinations were made between varieties both of which had tawny pubescence. In these crosses the factor for the production of anthocyanin may have been either $R_1$ or $R_2$ but for convenience $R_1$ has been used in case of doubt.

### DUPLICATE FACTORS FOR TAWNY PUBESCENCE

The assumptions that have just been made depend upon $T$ being the same factor for tawny pubescence in each case. It might have been assumed that there is only one factor $R$ for black seed-coat color and two factors for tawny pubescence. A convenient designation would be $T_1$ and $T_2$. The factor $T_1$ could be independent of $R$, but $R$ and $T_2$ would be completely linked. Such an assumption would explain the results as well as the method which has been chosen.

Further work is necessary to decide these relationships but the results from one $F_2$ population give a real indication for the presence of two factors for tawny pubescence. This was progeny No. 71 (No. Sp 3-9 × No. 29) which has been discussed under Part I of this series, as evidence for maternal inheritance of cotyledon color. Both paternal parents were homozygous for tawny pubescence but there was segregation in $F_2$ giving 71 tawny to 15 gray pubescent plants. Assuming duplicate factors the deviation is $10.6 \pm 4.8$. It is very evident that this is a rather wide deviation from a 15:1 ratio but since the odds are only about 6:1 it does not seem entirely improbable. $F_3$ progenies from this cross are to be grown and results reported at a future date.

## LINKAGE *versus* INDEPENDENCE OF SEVEN PAIRS OF FACTORS

Eleven crosses between Mandarins and an unidentified Chinese variety, given the arbitrary number C4, proved successful. These varieties differ in seven pairs of characters. These contrasted characters and the factors which correspond to them are as follows:

| *Mandarin* | *Green No. C4* |
|---|---|
| Purple flower color, $W$ | White flower color, $w$ |
| Yellow pods, $l$ | Black pods, $L$ |
| Gray pubescence, $t$ | Tawny pubescence, $T$ |
| Yellow seed-coat, $g$ | Green seed coat, $G$ |
| Light hilum, $I^h$ | Dark hilum, $I^i$ |
| Brown mottling pigment, $r_1$ | Black mottling pigment, $R_1$ |
| Yellow cotyledon color, $D_1D_2$ | Green cotyledon color, $d_1d_2$ |

The factor pairs $Gg$, $I^hI^i$, and $R_1r_1$ have the same meaning here as in NAGAI and SAITO'S (1923) descriptions; and $Ww$, $Ll$, $Tt$, and $D_1d_1$, have been described by WOODWORTH (1921 and 1923). The factor $D_2$ is a duplicate of $D_1$ for the production of yellow cotyledons, and is identical with WOODWORTH'S factor $I$, the only reason for the change being the use of $I$ in designating a restriction factor.

The action of $R_1$ in this cross was the same as has been previously described. The dark hilum was easily distinguished from light hilum on tawny-pubescent plants, but the intensity of pigment was reduced on gray-pubescent plants, making it difficult to detect the presence of the restriction factor. In fact, all the gray-pubescent plants bore seed so much lighter in color than the tawny-pubescent plants that all were classified as having light hilums. Therefore only 3/16 of the total number of plants should come in the dark hilum class, and a 13:3 ratio should be expected.

The seed-coat color due to plastid pigment was perhaps the most variable character. The $F_1$ seed coats produced a green color but it was somewhat intermediate. $F_2$ results were in very close agreement with a 3:1 ratio but there were all gradations in intensity of green. The pod color was no doubt responsible for much of this variation because light-colored pods were always associated with a "faded-out green." Perhaps this can be explained as a result of the plants remaining in the field in the presence of bright sunlight for a considerable length of time after ripening. Black-podded plants always produce a normal green or yellow seed coat, and it seems possible that this can be accounted for by attributing to the black color the absorption of the sunlight which changes normal green seed

coats to a yellowish green. In the light-colored pods the same effect was also produced on green cotyledons as described in Part I of this series.

There was a close approach to a 15:1 ratio for cotyledon color in all $F_2$ seed. The total numbers were 1686 with yellow cotyledons to 118 with green cotyledons, so the deviation is only $5.25 \pm 6.9$ from the expected numbers. Usually the seeds were easy to classify because the difference was clear-cut. Both of the parental varieties matured well in Wisconsin and no cotyledons were green due to immaturity. The $F_1$ plants also produced black pods, so there was no tendency for the cotyledon color to fade as it did on certain $F_2$ plants with yellow pods.

The parental variety No. C4 has very dark brown or black pods. As has been stated the pigment responsible for this coloration has no relation to the anthocyanin or the brown phlobaphene-like pigment; yet very frequently, on black-podded plants, much of this pigment finds its way into the seed coat and produces a smudgy-brown coloration. This condition has often masked other pigments to such an extent that the classification of seed-coat colors has been very difficult. Fortunately the $F_2$'s were grown on soil which brought out considerable mottling, but it was frequently necessary to treat the seed coats with acid in order to be sure of the classification. This test proved a great aid because a small amount of anthocyanin can be detected by the red color produced in acid solution.

TABLE 2

*$F_2$ progenies of Mandarin×Green No. C4.*

| | OBSERVED NUMBERS | DEVIATION | $\frac{\text{DEV.}}{\text{P. E.}}$ | ACTUAL RATIO |
|---|---|---|---|---|
| Green *versus* Yellow seed-coat | 252:85 | 0.75 | 0.14 | 2.96:1 |
| Tawny *versus* Gray pubescence | 262:75 | 9.25 | 1.73 | 3.49:1 |
| Anthocyanin *versus* Brown pigment | 251:86 | 1.75 | 0.33 | 2.92:1 |
| Black *versus* Yellow pods | 238:99 | 14.75 | 2.75 | 2.40:1 |
| Purple *versus* White flowers | 251:86 | 1.75 | 0.33 | 2.92:1 |
| Light *versus* Dark hilum (calculated on basis of 13:3) | 265:72 | 8.8 | 1.82 | 11.04:3 |
| Yellow *versus* Green cotyledons (corrected figures) | 321:16<br>315.9:21.1 | 5.1 | 1.70 | 20.06:1 |

Table 2 gives the observed data for each of the contrasted characters of 337 $F_2$ plants. There is good agreement with expectation among all seven of these contrasted characters when considered separately except black versus yellow pods. This discrepancy perhaps can be accounted for by the fact that the classification was made from a few pods after thresh-

ing. Since the character is somewhat variable, further tests would be necessary to prove that there is any other reason for the results not being in accord with a 3:1 ratio.

In order to determine whether or not there was any linkage, each contrasting pair of characters has been considered separately with every other pair. Table 3 gives these data in a condensed form. Twenty-one combinations are possible and the actual ratios as well as the theoretical are given in the table. The values of $\chi^2$ and P are also included.

TABLE 3

*$F_2$ progenies of Green No. C4×Mandarin—total of 337 plants.*

| | | | THEORETICAL RATIO WITH INDEPENDENCE | ACTUAL RATIO | $\chi^2$ | P |
|---|---|---|---|---|---|---|
| $D_1D_2$ | *versus* $d_1d_2$[1] | and *W versus w* | 45:15: 3:1 | 44.3:15.7: 3.5:0.5 | 2.07 | 0.56 |
| " | | and *T versus t* | 45:15: 3:1 | 46.2:13.8: 3.8:0.2 | 4.68 | 0.20 |
| " | | and $R_1$ *versus* $r_1$ | 45:15: 3:1 | 44.7:15.3: 3.0:1.0 | 0.05 | 0.9+ |
| " | | and *L versus l* | 45:15: 3:1 | 41.9:18.1: 3.5:0.5 | 6.45 | 0.09 |
| " | | and $I^h$ *versus* $I^i$ | 195:45:13:3[2] | 192.2:51.7: 9.1:3.0 | 1.83 | 0.61 |
| " | | and *G versus g* | 45:15: 3:1 | 44.1:15.9: 4.0:0.0 | 7.44 | 0.06 |
| *G* | *versus g* | and *W versus w* | 9: 3: 3:1 | 8.7: 3.2: 3.2:0.9 | 1.21 | 0.75 |
| " | | and *T versus t* | 9: 3: 3:1 | 9.1: 2.9: 3.3:0.7 | 2.69 | 0.45 |
| " | | and $R_1$ *versus* $r_1$ | 9: 3: 3:1 | 9.3: 2.7: 2.6:1.4 | 4.54 | 0.21 |
| " | | and *L versus l* | 9: 3: 3:1 | 8.7: 3.3: 2.6:1.4 | 5.58 | 0.14 |
| " | | and $I^h$ *versus* $I^i$ | 39:13: 9:3[2] | 37.6:12.7:10.3:3.4 | 1.52 | 0.68 |
| $I^h$ | *versus* $I^i$ | and *W versus w* | 39:13: 9:3 | 36.8:13.4:10.8:2.8 | 2.71 | 0.44 |
| " | | and *T versus t* | 9: 3: 4: | 9.0: 3.4: 3.6: | 2.25 | 0.33 |
| " | | and $R_1$ *versus* $r_1$ | 39:13: 9:3[2] | 36.6:13.6:11.0:2.7 | 3.50 | 0.33 |
| " | | and *L versus l* | 39:13: 9:3 | 35.5:14.8: 9.7:4.9 | 4.95 | 0.18 |
| *L* | *versus l* | and *W versus w* | 9: 3: 3:1 | 8.4: 2.9: 3.5:1.2 | 3.48 | 0.33 |
| " | | and *T versus t* | 9: 3: 3:1 | 8.7: 3.8: 2.6:0.9 | 5.30 | 0.15 |
| " | | and $R_1$ *versus* $r_1$ | 9: 3: 3:1 | 8.3: 3.6: 3.0:1.1 | 3.89 | 0.28 |
| $R_1$ | *versus* $r_1$ | and *W versus w* | 9: 3: 3:1 | 8.6: 3.3: 3.3:0.8 | 2.17 | 0.54 |
| " | | and *T versus t* | 9: 3: 3:1 | 9.4: 3.0: 2.5:1.0 | 2.07 | 0.56 |
| *T* | *versus t* | and *W versus w* | 9: 3: 3:1 | 9.3: 3.2: 2.6:0.9 | 1.41 | 0.71 |

[1] The observed ratio was corrected for a 15:1 ratio for cotyledon color in making comparisons with the calculated figures. This is legitimate and advisable because no effort was made to grow these seeds to maturity in exactly that proportion.

[2] These odd ratios are due to the fact that all gray-pubescent plants were classified as having light hilums. Really ¼ of these plants had dark hilums but they could not be readily identified.

It will be noted that five combinations give values of P less than 0.2 but four of these are combinations with black versus yellow pods where

the original ratio was 2.4:1; rather poor fits[1] with other pairs of characters, therefore, would naturally result. One combination alone (plastid color in the seed coat versus cotyledon color) appears to be a case of linkage. In this case the value of P is 0.06 which means that the odds are only about 16:1 against such a fit being due to chance but an examination of the actual results indicates that the odds may be greater. This linkage relation has been discussed in Part I of this series.

LINKAGES

| | |
|---|---|
| $R_2$, factor for black pigment in seed coat (Perhaps identical with WOODWORTH's *H*<br>*T*, factor for tawny pubescence. | Completely linked. |
| *T*, factor for tawny pubescence.<br>*E*, factor for early maturity. | Since the expression of *E* is somewhat quantitative the linkage could not be accurately measured, but there is proof for only a small percentage of crossing over. |
| $P_1$, factor for glabrousness.<br>$R_1$, factor for black pigment in seed coat. | Linkage appears to be almost complete with limited data. |
| $P_1$, factor for glabrousness.<br>*M*, factor for black mottling. | About 18 percent crossing over. (NAGAI and SAITO 1923) |
| *G*, factor for green seed coat, designated as *V* by WOODWORTH<br>$D_1$, factor for yellow cotyledon color | About 13 percent crossing over. (WOODWORTH 1921. Also the present study.) |

Three linkage groups are necessary to explain the above linkages, but the factors cannot be arranged according to their respective positions. These groups are as follows:

$$R_2 - T - E$$
$$P_1 - R_1 - M$$
$$G - D_1$$

LIST OF GENETIC FACTORS

The genetic factors used to explain the characters which have been studied in soybeans are as follows:

*T*, factor for tawny pubescence. Also increases the intensity of color in the seed coat; *t*, gray pubescence and a lighter seed-coat color. (WOODWORTH 1921) (Limited evidence indicates that there may be another factor which produces the same results as *T* but is independently inherited.)

*W*, factor for purple flower and hypocotyl color and also a complement of $r_2$ for the production of black pigment in the seed coat; *w*, white flowers and green hypocotyls. (WOODWORTH 1923.)

[1] A preferable method of calculating $x^2$ as a measure of linkage follows this article.

$R_1$, factor for black pigment in the seed-coat but without $T$ the color is imperfect black; $r_1$, brown pigment but without $T$ the color is light brown or buff. (NAGAI 1921.)

$R_2$, also a factor for black pigment in seed coat, but entirely linked with $T$ (possibly this factor is identical with WOODWORTH's factor $H$); $r_2$, brown pigment (but is a complement of $W$. With $r_2r_2$ and $W$ present, the pigment is imperfect black in appearance); $r_2'$, brown pigment but not complementary to $W$.

$B$, found to be a complementary factor to Woodworth's $H$ for the production of black pigment in the seed-coat. (WOODWORTH 1921.)

$O$, factor for dull brown seed-coat color; $o$, reddish brown. (NAGAI 1921.)

$I$, factor which inhibits pigment formation in the seed-coat—an allelomorphic series. $i$, has no effect.

- $I^k$, inhibits pigment to form an eyebrown pattern (identical with NAGAI and SAITO's, 1923, factor $K$).
- $I^i$, inhibits pigment to the hilum—dominant to $I^k$ and $i$. (Possibly the same as the factor $I$ which NAGAI, 1921, describes as nearly completely inhibiting the formation of chromogenic substance in the seed-coat.)
- $I^h$, inhibits pigments entirely except that which may be due to mottling—dominant to $I^i$, $I^k$, and $i$. (Identical with NAGAI and SAITO's factor $H$.)

$M$, factor for the production of black mottling upon a self-brown seed-coat; $m$, no effect. (NAGAI and SAITO 1923.)

$G$, factor for green seed-coat; $g$, yellow seed coat due to the green plastid color fading out at maturity. (NAGAI, 1921, also WOODWORTH, 1921, but $V$ was used for the symbol instead of $G$.)

$D_1$, factor for yellow cotyledon color; $d_1$, no effect. (WOODWORTH 1921.)

$D_2$, duplicate of $D_1$, for causing the green cotyledon color to fade out at maturity (identical with WOODWORTH's factor $I$); $d_2$, no effect.

$N$, factor for normal hilum; $n$, abnormal hilum as in the Soysota variety.

$P_1$, factor for glabrousness; $p_1$, pubescence. (NAGAI and SAITO 1923.)

$P_2$, factor for pubescence, $p_2$, glabrousness (relationship to $P_1$ and $p_1$ not known as yet except that the order of dominance is reversed)—STEWART and WENTZ (1926).

$L$, factor for black pods but also causes a black coloration of the seed-coat in certain instances; $l$, brown pods. (WOODWORTH 1923.)

$S$, factor for tall plant; $s$, short plant. (WOODWORTH, 1923, also reported by the writer in table 8.)

$E$, factor for early maturity; $e$, late maturity.

In the above list of factors reference is made to all original descriptions of previous investigators. Those factors which do not have such a reference are described here for the first time. Of course, there is no definite proof that the factors which are considered synonymous are really identical, but it seems advisable to consider them as such until there is proof for the contrary.

## SUMMARY

In all the crosses that have been made black seed coat has been dominant to brown but in one cross the dominance was incomplete. Results also indicate that different factors may produce black pigment; and a genetic interpretation has been given to account for the presence of $R_1$

and $R_2$, each of which is capable of producing anthocyanin pigment in the seed coat.

In one particular cross, the factor $W$, for purple flowers, was found to be a complement of $r_2$ for the production of black pigment in the seed coat; but in all other cases purple-flower color was independent of every other character.

Three restriction factors have been described. These are dominant factors but their expression is very sensitive to the conditions under which the plant is grown. An environment unfavorable for the production of mottling is necessary for pigment to be completely restricted; otherwise there may be considerable mottling in spite of the presence of restriction factors.

The factor $T$, for tawny pubescence, has proved to be very important in increasing the intensity of pigment. This is perhaps due to an actual increase in the amount of pigment.

In black-podded varieties a certain amount of the brownish-black oxidation pigment of the pod was sometimes evident in the seed coat.

Five linkages are postulated. The crossover percentage between $G$, the factor for green seed coat, and $D$, a factor for yellow cotyledons, was approximately 13 percent, the same as WOODWORTH (1921) has reported. The factor $T$, for tawny pubescence was completely linked to $R_2$. All other linkages are quite tentative until more data are secured.

A complete list of all the genetic factors studied in soybeans has been given as well as those directly concerned with seed-coat colors.

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## APPENDIX

### TABLE 4

*$F_2$ progenies of brown ($r_1i$)×yellow with black hilum ($R_1$ $I^h$).*

| | PROGENY NUMBERS | DARK HILUM | | SELF-COLORED SEED-COAT | | |
|---|---|---|---|---|---|---|
| | | Black $R_1I^i$ | Brown $r_1I^i$ | Black $R_1i$ | Brown $r_1i$ | |
| Soysota (Brown No. 30) ×Manchu (No. 59) | 213 | 58 | 14 | 18 | 9 | $\chi^2=2.53$ |
| | 224 | 53 | 13 | 13 | 6 | $P=0.47$ |
| | 217 | 84 | 28 | 33 | 13 | |
| | Total | 195 | 55 | 64 | 28 | |
| *Calculated* | | *192.4* | *64.1* | *64.1* | *21.4* | |
| Difference | | +2.6 | −9.1 | −0.1 | +6.6 | |
| Brown (No. 12)×Manchu (No. 1) | 186 | 71 | 11 | 18 | 5 | $\chi^2=4.09$ |
| | 190 | 128 | 41 | 38 | 11 | $P=0.25$ |
| | Total | 199 | 52 | 56 | 16 | |
| *Calculated* | | *181.7* | *60.6* | *60.6* | *20.2* | |
| Difference | | +17.3 | −8.6 | −4.6 | −4.2 | |
| Brown (No. 12)×Manchu (No 16) *Calculated* | 8 | 190 | 62 | 51 | 27 | $\chi^2=4.95$ |
| | | *185.6* | *61.9* | *61.9* | *20.6* | $P=0.18$ |
| Difference | | +4.4 | +0.1 | −10.9 | +6.4 | |
| Total of $F_2$ progenies | | 584 | 169 | 171 | 71 | $\chi^2=5.26$ |
| *Calculated* | | *559.7* | *186.6* | *186.6* | *62.2* | $P=0.16$ |
| Difference | | +24.3 | −17.6 | −15.6 | +8.8 | |

TABLE 5

*$F_2$ progenies of yellow ($tI^k$)×brown seed coat ($Ti$).*

| | PROGENY NUMBERS | TAWNY PUBESCENCE | | GRAY PUBESCENCE | | |
|---|---|---|---|---|---|---|
| | | Mottled with brown | Self-brown | Mottled with brown | Self-brown | |
| Mandarin×Brown (No. 12) | 197 | 40 | 10 | 10 | 6 | $\chi^2=2.04$ |
| *Calculated* | | *37.1* | *12.4* | *12.4* | *4.1* | $P=0.57$ |
| Difference | | +2.9 | −2.4 | −2.4 | +1.9 | |
| Mandarin×Soysota (Brown No. 30) | 231 | 10 | 2 | 5 | 3 | $\chi^2=3.61$ |
| *Calculated* | | *11.3* | *3.7* | *3.7* | *1.3* | $P=0.31$ |
| Difference | | −1.3 | +1.7 | +1.3 | +1.7 | |
| Yellow (No. C2)×Soysota (Brown No. 30) | 208 | 68 | 26 | 18 | 9 | $\chi^2=1.71$ |
| *Calculated* | | *68* | *22.7* | *22.7* | *7.6* | $P=0.65$ |
| Difference | | | +3.3 | −4.7 | +1.4 | |
| Aksarben×Brown (No. 60) | 164a | 19 | 10 | 5 | 4 | $\chi^2=3.14$ |
| *Calculated* | | *21.4* | *7.1* | *7.1* | *2.4* | $P=0.37$ |
| Difference | | −2.4 | +2.9 | −2.1 | +1.6 | |
| Total observed | | 137 | 48 | 38 | 22 | $\chi^2=4.39$ |
| *Calculated* | | *137.8* | *45.9* | *45.9* | *15.3* | $P=0.23$ |
| Difference | | −0.8 | +2.1 | −7.9 | +6.7 | |

TABLE 6

*Dark black hilum* ($R_1I^i$)×*light brown hilum* ($r_1I^h$).

| | NUMBER OF PROGENIES | LIGHT HILUM | | DARK HILUM | | |
|---|---|---|---|---|---|---|
| | | Black pigment | Brown pigment | Black pigment | Brown pigment | |
| $F_2$ Progeny No. 1 (Manchu No. 1×Yellow No. 12) | | 120 | 36 | 51 | 14 | $\chi^2=3.10$ |
| | | *124.4* | *41.4* | *41.4* | *13.8* | $P=0.38$ |
| | | −4.4 | −5.4 | +9.6 | +0.2 | |
| $F_3$ Progenies from seeds with light black hilum | 1 | 45 | 12 | 16 | 2 | |
| Deviation from calculated | | +2.8 | −2.1 | +1.9 | −2.7 | |
| | 1 | 36 | | 14 | | |
| Deviation from calculated | | −1.5 | | +1.5 | | |
| | 2 | 49 | 18 | | | |
| Deviation from calculated | | −1.3 | +1.3 | | | |
| | 1 | 17 | | 7 | | |
| Deviation from calculated | | −1 | | +1 | | |
| | 2 | 57 | | | | |
| $F_3$ Progenies from seeds with black dark hilum | 1 | | | 11 | 2 | |
| Deviation from calculated | | | | +1.2 | −1.3 | |
| | 2 | | | 76 | | |
| $F_3$ Progenies from seeds with brown light hilum | 3 | | 71 | | 26 | |
| Deviation from calculated | | | +1.8 | | −1.7 | |
| | 2 | | 38 | | | |
| $F_3$ Progenies from seeds with dark brown hilum | 2 | | | | 44 | |

TABLE 7

*Tawny-pubescent, Dark black hilum ($tI^{t}R_{1}$)×Gray-pubescent, Light brown hilum ($tI^{b}r_{1}$).*

| | PROGENY NUMBERS | TAWNY PUBESCENCE | | | | GRAY PUBESCENCE | | |
|---|---|---|---|---|---|---|---|---|
| | | Light Hilum | | Dark Hilum | | Light-dark Hilum | | |
| | | Black Pigment | Brown Pigment | Black Pigment | Brown Pigment | Black Pigment | Brown Pigment | |
| Green (No. C4)×Aksarben | 31 | 20 | 6 | 5 | 3 | 5 | 6 | |
| Deviation from calculated | | +1.0 | −0.3 | −1.3 | +0.9 | −3.4 | +3.2 | $\chi^2=5.75$ $P=0.33$ |
| Yellow (No. C2)×Manchu (No. 1) | 269 | 10 | 4 | 6 | 4 | 11 | 2 | |
| Deviation from calculated | | −5.6 | −1.2 | +0.8 | +2.3 | +4.1 | −0.3 | $\chi^2=8.00$ $P=0.16$ |
| Mandarin×Green (No. C1) | 342 | 7 | 6 | 9 | 2 | 7 | 2 | |
| Deviation from calculated | | −6.9 | +1.4 | +4.4 | +0.5 | +0.9 | −0.1 | $\chi^2=8.33$ $P=0.14$ |
| Mandarin×Manchu (No. 1) | 300 | 161 | 62 | 40 | 14 | 51 | 20 | |
| | 292 | 14 | 6 | 4 | 1 | 5 | 1 | |
| | Total | 175 | 68 | 44 | 15 | 56 | 21 | |
| Deviation from calculated | | +15.1 | +14.7 | −9.3 | −2.8 | −15.1 | −2.7 | $\chi^2=11.06$ $P=0.05$ |
| Manchu (No. 59)×Mandarin | 282 | 134 | 47 | 48 | 17 | 71 | 23 | |
| | 272 | 28 | 14 | 7 | 3 | 9 | 11 | |
| | Total | 162 | 61 | 55 | 20 | 80 | 34 | |
| Deviation from calculated | | −11.8 | +3.1 | −2.9 | +0.7 | +2.7 | +8.2 | $\chi^2=3.84$ $P=0.57$ |
| Green (No. C4)×Mandarin | 13 | 140 | 50 | 58 | 14 | 53 | 22 | |
| Deviation from calculated | | −2.2 | +2.6 | +10.6 | −1.8 | −10.2 | +0.9 | $\chi^2=4.44$ $P=0.49$ |
| *Calculated on basis of 27:9:9:3:12:4 ratio* | Total | 514 | 195 | 177 | 58 | 212 | 87 | |
| | | *524.5* | *174.8* | *174.8* | *58.3* | *233.1* | *77.7* | |
| Difference | | −10.5 | +20.2 | +2.2 | −0.3 | −21.1 | +9.3 | $\chi^2=5.60$ $P=0.35$ |

TABLE 8

*Showing independence between S, the dominant factor for height of plant and $R_1$ and G—Progeny No. 319—No. 57 ($Sr_1g$)×No. $C_1$ ($sR_1G$).*

| | $SGR_1$ | $SGr_1$ | $SgR_1$ | $Sgr_1$ | $sGR_1$ | $sGr_1$ | $sgR_1$ | $sgr_1$ | |
|---|---|---|---|---|---|---|---|---|---|
| Observed | 85 | 29 | 24 | 9 | 33 | 8 | 11 | 3 | $\chi^2$=1.95 |
| *Calculated* | *85.2* | *28.4* | *28.4* | *9.5* | *28.4* | *9.5* | *9.5* | *3.2* | *P*=0.96 |
| Deviation | −.2 | +.6 | −4.4 | −.5 | +4.6 | −1.5 | +1.5 | −.2 | |

TABLE 9

*Manchu ($R_1I^iN$)×Soysota ($r_1in$).*

| | $NR_1I^i$ | $NR_1i$ | $Nr_1I^i$ | $Nr_1i$ | $nR_1I^i$ | $nR_1i$ | $nr_1I^i$ | $nr_1i$ | |
|---|---|---|---|---|---|---|---|---|---|
| Observed | 41 | 11 | 8 | 5 | 17 | 7 | 6 | 4 | $\chi^2$=9.70 |
| *Calculated* | *41.8* | *13.9* | *13.9* | *4.6* | *13.9* | *4.6* | *4.6* | *1.5* | *P*=0.22 |
| Difference | −0.8 | −2.1 | −5.9 | −0.4 | +3.1 | +2.4 | +1.4 | +2.5 | |

TABLE 10

*Black No. 3 ($R_3N$)×Soysota ($r'_3n$).*

| | $R_3N$ | $R_3n$ | $r_3N$ | $r_3n$ | |
|---|---|---|---|---|---|
| Observed | 49 | 23 | 21 | 7 | |
| *Calculated* | *56.25* | *18.75* | *18.75* | *6.25* | $\chi^2$=2.26 |
| Difference | −7.25 | +4.25 | +2.25 | +0.75 | *P*=0.50 |

# CALCULATING LINKAGE INTENSITIES BY PRODUCT MOMENT CORRELATION[1]

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## INTRODUCTION

The need for a reliable method of calculating linkage intensities from $F_2$ populations is apparent from the literature and is appreciated after a minimum of experience. The present contribution is an attempt to make use of well known statistical work by illustrating the relationship between product moment correlation and crossing over. For "theoretical Mendelism" there is no doubt that the Pearsonian product moment coefficient of correlation may be used with entire safety. The "Pearsonian school" (PEARSON and HERON 1913) grants this usage but objects when it is applied to "practical Mendelism."

The whole crux of the matter, therefore, lies in the interpretation of "practical Mendelism." Are phenotypic classes so confluent that gametic values can never be considered as discrete units? One must candidly admit that it all depends upon the particular unit character under consideration, the environment under which it is produced (MORGAN 1919,

[1] Papers from the Biological Laboratory, MAINE AGRICULTURAL EXPERIMENT STATION, No. 180.

page 29) and the skill of the investigator (EMERSON 1921, page 16). The methods of calculating linkage intensities here presented presuppose clear-cut Mendelian segregation, and it should be perfectly obvious that as the classifications become increasingly arbitrary that estimations of linkage intensities become more and more inaccurate.

COLLINS (1924) has described three methods of measuring linkage (EMERSON'S, HALDANE'S and YULE'S) all of which give conflicting results when the observed ratios do not agree exactly with the theoretical. EMERSON'S (1916) method is simple and a table has been worked out by ALBERTS (1926) but the calculations are not based on random deviations from the 3:1 ratio. HALDANE'S (1919) method is valuable but the end results are not always satisfactory. COLLINS maintains that YULE'S coefficient of association $Q$ "most nearly meets the requirements of a general method" but "no one (of the three methods) is found to be most accurate for all classes of departures." More recently FISHER (1925) has outlined a method based upon the best possible fit in comparison with theoretical ratios. Fundamentally the procedure is the same as HALDANE'S but FISHER'S formula is much more convenient for application. This method is especially valuable but is subject to the same criticism which COLLINS has given for HALDANE'S method unless it is accompanied by additional information.

It is the writer's intention that the method which is being introduced should, for certain critical cases, be accompanied by the use of FISHER'S and YULE'S formulae, but for a general method the product moment coefficient of correlation seems the most logical. Not only are the results more consistent and conservative but one gains the additional advantage of convenient algebraic manipulation.

For a $2\times2$ classification the product moment coefficient of correlation (made possible by assuming clear-cut Mendelian characters) is identical with the square root of the mean square contingency. Thus the simple relationship $\chi^2=nr^2$, since the mean square contingency $\phi^2=\frac{\chi^2}{n}$. This makes it possible to estimate the crossing over percentage from $r$ and also the odds for goodness of fit by $\chi^2$ in one operation. The simple relationship minimizes the labor of calculation and gives a critical test. Furthermore, the method is not confined to correlation between characters determined by single factors but has been readily extended to duplicate and complimentary factors and various other complex relationships and also to simple cases of backcrossing.

It is hoped that the tables[1] which have been prepared from theoretical ratios will serve both as a convenience and a caution. Furthermore the basis for assuming a product moment coefficient of correlation must be constantly kept in mind. When practical observations depart from the theoretical extreme care must be used and a wide field (see KELLEY 1924, page 259) is open for the development of statistical criteria.

## THE PRODUCT MOMENT COEFFICIENT OF CORRELATION

A purely theoretical $F_2$ distribution where $AB=a''$, $Ab=b''$, $aB=c''$, and $ab=d''$ may be arranged in the fourfold table:

| | $A$ | $a$ | |
|---|---|---|---|
| $B$ | $a''$ | $b''$ | $a''+b''$ |
| $b$ | $c''$ | $d''$ | $c''+d''$ |
| | $a''+c''$ | $b''+d''$ | $n$ |

Without any knowledge as to the definite value of the qualitative categories $A$, $a$, $B$ and $b$, and without any regard as to the observed ratios or the position of the means, the Pearsonian product moment coefficient of correlation turns out:

$$r^2=\frac{(a''d''-b''c'')^2}{(a''+b'')(c''+d'')(a''+c'')(b''+d'')} \tag{1}$$

by simple substitution in the general equation

$$r=\frac{\Sigma XY-nM_xM_y}{\sqrt{(\Sigma X^2-nM_x^2)(\Sigma Y^2-nM_y^2)}}$$

Since the assumptions are purely theoretical there can be no objection to considering $A$, $a$, $B$, and $b$ as definite abstract values, that is they do not spread over an interval. Hence there is perfect justification for deriving this coefficient of correlation. For critical references see KELLEY 1924, page 259; PEARSON and HERON 1913, page 167; BOAS 1909; YULE 1912; and SNOW 1912.

### *Relation to $\chi^2$ test for goodness of fit*

When $r=0$ it may be assumed that $a''=a'''$, $b''=b'''$, $c''=c'''$ and $d''=d'''$. Then applying the test for goodness of fit

[1] These tables were calculated by Mrs. IVA MERCHANT BURGESS and Miss MILDRED COVELL of the Biological Laboratory of the MAINE AGRICULTURAL EXPERIMENT STATION.

$$\chi^2 = \frac{(a''-a''')^2}{a'''} + \frac{(b''-b''')^2}{b'''} + \frac{(c''-c''')^2}{c'''} + \frac{(d''-d''')^2}{d'''}$$
$$= \frac{(a''d''-b''c'')^2\,(a''+b''+c''+d'')}{(a''+b'')(c''+d'')(a''+c'')(b''+d'')} \qquad (2)$$
$$= nr^2$$

This is but a simple illustration of the well recognized value of $\chi^2$ when the fourfold table is made up according to the specifications of theoretical Mendelism. (See previous references to KELLEY, and PEARSON and HERON).

If, however, the observed values of $AB$, $Ab$, $aB$ and $ab$ are not in approximate agreement with the theoretical, that is, when $A$, $a$, $B$, and $b$ fluctuate to such an extent that the whole distribution must be thought of as continuous; then the statistical analysis is very different. The well known tetrachoric $r_t$ has been devised for these cases and the value $\frac{\chi^2}{n}$ is equal to the uncorrected mean square contingency $\phi^2$, but with continuous variation $\phi$ cannot be considered synonymous with the product moment coefficient of correlation.

### *Determining odds against random distribution*

For determining the goodness of fit COLLINS (1924) has already suggested that observed data should be compared with a fourfold distribution based on the observed rather than the theoretical ratios of dominant to recessive phenotypes. This is true if $\chi^2$ is to be a measure of correlation.

To clarify terminology the following definitions may be considered:

| | | PHENOTYPIC DISTRIBUTION | | | |
|---|---|---|---|---|---|
| | | $AB$ | $Ab$ | $aB$ | $ab$ |
| Observed ratios maintained | Any specific value of $r$ | $a$ | $b$ | $c$ | $d$ |
| | Correlation reduced to zero | $a'$ | $b'$ | $c'$ | $d'$ |
| Ratios strictly theoretical (3:1 or 9:7; etc.) | Any specific value of $r$ | $a''$ | $b''$ | $c''$ | $d''$ |
| | Correlation reduced to zero | $a'''$ | $b'''$ | $c'''$ | $d'''$ |

For observed data the fourfold table is:

| | | |
|---|---|---|
| $a$ | $b$ | $a+b$ |
| $c$ | $d$ | $c+d$ |
| $a+c$ | $b+d$ | $n$ |

and the ratios are $\frac{a+b}{n} : \frac{c+d}{n}$ and $\frac{a+c}{n} : \frac{b+d}{n}$

In the simplest case both of these ratios are approximately 3:1 but much recent evidence (See JONES 1924) clearly shows that various ratios are possible depending upon differential viability of gametes and selective pollen tube growth.

Certainly a comparison with the observed ratios is the more logical method for determining correlation and estimating linkage values. Furthermore such a comparison can be readily made. In fact this is automatically taken care of either in calculating $r$, the coefficient of correlation or YULE'S $Q$, the coefficient of association. To determine the uncorrelated distribution which does not change the ratios one must have the following equalities: $a+b=a'+b'$, $c+d=c'+d'$, $a+c=a'+c'$ and $b+d=b'+d'$. Hence,

$$a' = \frac{(a+b)(a+c)}{n}$$

$$b' = \frac{(a+b)(b+d)}{n}$$

$$c' = \frac{(c+d)(a+c)}{n}$$

$$d' = \frac{(c+d)(b+d)}{n}$$

In practise, however, it is necessary to calculate $a'$ only, since the other values can be obtained directly by subtraction in the equalities just given. (See FISHER 1925, page 85).

If it is possible to consider the observed frequencies as discrete units, the product moment coefficient of correlation may be calculated from formula [1] and $nr^2 = \chi^2$. Now the customary practise has been to consult ELDERTON'S tables (PEARSON 1914) to ascertain the probability of observed values of $\chi^2$. No criticism is intended for this procedure but one must remember that the probability secured in this way represents the

odds against a fit as bad as the observed being due to fluctuations in sampling. Obviously a perfect fit is as highly improbable as an extremely bad fit. To ascertain the odds against $\chi^2$, as a measure of correlation, being due to chance is a very different problem. (See KELLEY 1924, page 264).

If it is desired to estimate the odds against random distribution the standard deviation of $r$ deserves serious consideration. This standard deviation is (See YULE 1912, and PEARSON and HERON 1913, page 169)

$$\sigma_r=\frac{1}{\sqrt{n}}\left\{1-r^2+\left(r+\frac{r^3}{2}\right)\left(\sqrt{\frac{q_1}{p_1}}-\sqrt{\frac{p_1}{q_1}}\right)\left(\sqrt{\frac{q_2}{p_2}}-\sqrt{\frac{p_2}{q_2}}\right)-\frac{3r^2}{4}\left(\frac{q_1}{p_1}+\frac{p_1}{q_1}-2\right)\left(\frac{q_2}{p_2}+\frac{p_2}{q_2}-2\right)\right\}^{\frac{1}{2}} \tag{3}$$

where $p_1$, $q_1$, $p_2$ and $q_2$ are the observed ratios.

For backcrossing (with theoretical 1:1 and 1:1 ratios) this formula reduces to

$$\sigma_r=\frac{\sqrt{1-r^2}}{\sqrt{n}}$$

and in an ordinary $F_2$ (with 3:1 and 3:1 ratios)

$$\sigma_r=\frac{\sqrt{1+4/3r-7/3r^2+2/3r^3}}{\sqrt{n}}$$

It is obvious that this standard deviation reduces to $\frac{1}{\sqrt{n}}$ for all possible ratios when $r=0$. For genetic interpretations this is another point of considerable interest because ${}_o\sigma_r$, the standard deviation of $r$ for a random distribution, is the value which is generally desired. It is of further interest that $\frac{r}{{}_o\sigma_r}=\chi=\frac{Q}{{}_o\sigma_q}$ (See PEARSON 1912). This value $\chi$ is actually the square root of $\chi^2$ so the convenience is at once apparent. The only unfortunate thing is that the frequency of $r$ in terms of ${}_o\sigma_r$ (the standard deviation with random distribution) probably cannot be considered normal or Gaussian. PEARSON (1912) has given the probability integral for the desired frequency distribution but this awaits further study and development. It is hoped that eventually tables for this probability integral will be available. Then the odds against random distribution can be looked up directly rather than using the probability for goodness

of fit from ELDERTON's tables. The odds for goodness of fit from ELDERTON's tables (see COLLINS 1924, for table of odds) are very convenient but one must keep in mind that the odds against $\chi^2$ as a measure of correlation (not goodness of fit) may be very much greater.

*Relation to percentage crossing over*

For theoretical distributions there is fortunately a very simple relationship between the coefficient of correlation of zygotic phenotypes and the crossing over percentage of the two genetic factors under consideration. In the male the frequency of the $AB$ or $ab$ gametes may be represented by $p$ and the $Ab$ or $aB$ gametes by $(1-p)$ if conditions are normal. Likewise in the female let $p'$ represent the $AB$ or $ab$ gametes and $(1-p')$ the $Ab$ or $aB$ gametes. This leads to the following comparison in the $F_2$ generation.

| $AB$ | $Ab$ | $aB$ | $ab$ |
|---|---|---|---|
| $2+pp'$ | $1-pp'$ | $1-pp'$ | $pp'$ |

If one can further assume that the crossing over in the female has been the same as that in the male the problem becomes still more simple, that is, $pp'=p^2$.

Now calculating the coefficient of correlation from formula [1]

$$r=\frac{4p^2-1}{3} \text{ or } p=\frac{\sqrt{3r+1}}{2}$$

With similar assumptions (that $p=p'$ etc.) it is possible to estimate the crossing over percentage from observed data for various factorial relationships.

The modification of formula [1], suggested by KELLEY (1924, page 259) is preferable for this purpose. From the definitions

$$p_1=\frac{a+b}{n}, \qquad q_1=\frac{c+d}{n}, \qquad p_2=\frac{a+c}{n} \text{ and } q_2=\frac{b+d}{n}$$

$$r=\frac{d-q_1q_2}{\sqrt{p_1p_2q_1q_2}} \tag{4}$$

The simplicity of this formula should be appreciated by students of genetics because it brings out the relationship between the coefficient of correlation and percentage crossing over for various complex sorts of Mendelian combinations. Furthermore it illustrates a convenient method of building up a theoretical distribution when only the phenotypic ratios and the coefficient of correlation are known, because

$$d = r\sqrt{p_1 p_2 q_1 q_2} + q_1 q_2 \qquad (5)$$

and since $q_1 = \frac{c+d}{n}$, etc., $c$, $b$ and $d$ can be calculated very readily.

Table 1 gives the values of $p$ (proportion of $AB$ or $ab$ gametes) in terms of $r$ for the Mendelian combinations which are most likely to be considered. The less practical formulae, (COLLINS 1924) calculated directly according to EMERSON'S (1916) original method, are also included.

TABLE 1

*Crossing over percentage from coefficient of correlation compared with direct calculation.*

| THEORETICAL PHENOTYPIC RATIOS | VALUE OF $p^2$ FROM DIRECT CALCULATION | VALUE OF $p^2$ FROM COEFFICIENT OF CORRELATION |
|---|---|---|
| 1:1 and 1:1 (Backcross) | $\left(\frac{a+d}{n}\right)^2$ | $\frac{(r+1)^2}{4}$ |
| 3:1 and 3:1 (No crossing over in one sex)[1] | $\left(\frac{2(a+d)}{n}-1\right)^2$ | $\frac{(3r+1)^2}{16}$ |
| 3:1 and 3:1 | $\frac{2(a+d)}{n}-1$ | $\frac{3r+1}{4}$ |
| 15:1 and 3:1 | $\frac{8(a+d)}{n}-5.5$ | $\frac{3\sqrt{5}r+1}{4}$ |
| 63:1 and 3:1 | $\frac{32(a+d)}{n}-23.5$ | $\frac{3\sqrt{21}r+1}{4}$ |
| 15:1 and 15:1 | $\frac{32(a+d)}{n}-28$ | $\frac{15r+1}{4}$ |
| 9:7 and 3:1 | $\frac{16(a+d)-7n}{6n}$ | $\frac{\sqrt{21}r+1}{4}$ |
| 27:37 and 3:1 | $\frac{64(a+d)-25n}{18n}$ | $\frac{\sqrt{37}r+1}{4}$ |
| 9:7 and 9:7 | $\frac{32(a+d)-14n}{9n}$ | $\frac{7r+1}{4}$ |
| 9:7 and 15:1 | $\frac{32(a+d)-17n}{3n}$ | $\frac{\sqrt{105}r+1}{4}$ |

[1] All other formulae are based on equal crossing over percentages in both sexes.

As a practical procedure $r$ may be calculated from an observed distribution if the characters are clear-cut. If the ratios are abnormal, however, it is obvious that a value of $p$ for a theoretical distribution corresponding to this value of $r$ may not be the true value of $p$, but it should be a very good approximation. In any event it represents the same amount of

correlation in a theoretical distribution that is implied by the value of $r$ derived from the observed distribution. It is doubtful if a better assumption can be made unless there is knowledge of some abnormality. Furthermore the value of $r$ is conservative and for all the abnormal deviations due to differential mortality of gametes or zygotes calculated in tables 2, 3 and 4, $r$ does not indicate more linkage than that implied by the *a priori* crossover values.

## COMPARISON OF VARIOUS METHODS OF ESTIMATING LINKAGE VALUES

The methods of estimating linkage values may be grouped into two general classes, 1st, those which make direct comparisons with theoretical ratios—FISHER'S, HALDANE'S and EMERSON'S. 2nd, those which make comparisons with observed ratios—YULE'S coefficient of association $Q$ and the coefficient of correlation $r$[1].

FISHER'S and HALDANE'S methods give practically identical results as should be expected because they are based upon the same principles but the former is more simply applied. For theoretical ratios EMERSON'S method gives the same results as all other methods but it is not well adapted to practical use where slight deviations from the theoretical results are of common occurrence.

The coefficient of correlation $r$ is a more stable and reliable measure of correlation than the coefficient of association $Q$ and is therefore more adaptable for general use. However, $Q$ is especially useful when there is differential death rate of zygotes (See also COLLINS 1924) and should probably be calculated simultaneously with $r$ as a general practise. In fact this is especially convenient because of the similarity of the two formulae, [1] and [7].

For a comparison of these five methods see tables 2, 3 and 4 which were first suggested by COLLINS (1924).

### *Fisher's method*

FISHER (1925, page 25) has called attention to a very ingenious method of deriving a theoretical $F_2$ distribution which gives the best fit in comparison with the observed distribution. It is a simple method with all the good qualities of HALDANE'S $T$ (1919) and may well replace the original formula suggested by EMERSON (1916).

FISHER'S method is based upon the value of $p$ which makes $\chi^2$ a minimum. This is readily accomplished by multiplying the observed frequen-

[1] Termed the Boas-Yulean $\phi$ by PEARSON and HERON (1913).

cies by the logarithm of their theoretical functions and finding the value of $p$ which makes the sum of these products a maximum. The necessary differentiation and algebraic transformation makes it possible to write

$$p^2=\frac{(a-2b-2c-d)+\sqrt{(a-2b-2c-d)^2+8dn}}{2n} \qquad (6)$$

for an $F_2$ distribution (with 3:1 and 3:1 ratios). If there is no crossing over in one sex a similar procedure gives

$$p=\frac{(a-2b-2c-d)+\sqrt{(a-2b-2c-d)^2+8dn}}{2n}$$

and for the simple case of backcrossing (with 1:1 and 1:1 ratios)

TABLE 2

*Comparison of methods of measuring linkage values in a backcross*— (1:1 *and* 1:1 *ratios*).

| | ZYGOTIC CLASSES—IN PERCENT | | | | OBSERVED VALUES OF $p^2$ FROM: | | |
|---|---|---|---|---|---|---|---|
| | *a* | *b* | *c* | *d* | EMERSON'S[2] $p$ | YULE'S $Q$ | $r$ |
| No actual gametic linkage | | | | | | | |
| Perfect population | 25.00 | 25.00 | 25.00 | 25.00 | 50.00 | 50.00 | 50.00 |
| 25 percent *a* gametes not effective | 28.57 | 28.57 | 21.43 | 21.43 | 50.00 | 50.00 | 50.00 |
| 25 percent *a* and *b* gametes not effective | 32.65 | 24.49 | 24.49 | 18.37 | 51.02 | 50.00 | 50.00 |
| 25 percent *a* and *B* gametes not effective | 24.49 | 32.65 | 18.37 | 24.49 | 48.98 | 50.00 | 50.00 |
| 25 percent *a* zygotes wrongly classified as *A* | 31.25 | 31.25 | 18.75 | 18.75 | 50.00 | 50.00 | 50.00 |
| 25 percent actual gametic linkage—Coupling phase[1] | | | | | | | |
| Perfect population | 37.50 | 12.50 | 12.50 | 37.50 | 75.00 | 75.00 | 75.00 |
| 25 percent *a* gametes not effective | 42.86 | 14.29 | 10.71 | 32.14 | 75.00 | 75.00 | 74.81 |
| 25 percent *a* and *b* gametes not effective | 48.48 | 12.12 | 12.12 | 27.27 | 75.77 | 75.00 | 74.61 |
| 25 percent *a* and *B* gametes not effective | 37.11 | 9.28 | 16.49 | 37.11 | 74.24 | 75.00 | 74.61 |
| 25 percent *a* zygotes wrongly classified as *A* | 40.63 | 21.88 | 9.38 | 28.13 | 68.75 | 79.76 | 69.37 |

[1] For coupling phase the crossing over percentage is equal to $1-p$.

[2] EMERSON'S direct method gives the closest fit to the theoretical ratios for backcrosses and may, therefore, be considered synonymous with FISHER'S and HALDANE'S methods.

$$p = \frac{a+d}{n}$$

the direct value which has been customarily used.

More complex relations may also be treated in a similar manner. Unfortunately, it is necessary to solve a cubic equation unless two of the theoretical classes are identical.

As long as the observed distribution can be thought of as a normal deviation from the theoretical, there is good justification for FISHER'S method and a crossing over percentage calculated in this way is a very good approximation to that which really takes place. However, it may frequently be desirable to know the actual correlation as indicated by the coefficient of correlation because linkage (or correlation) may be implied by FISHER'S method when actually none exists (tables 2, 3 and 4.) See COLLINS, 1924, for a similar criticism of HALDANE'S method.

TABLE 3

*Comparison of methods of measuring linkage values in $F_2$—no actual gametic linkage.*

| | ZYGOTIC CLASSES—IN PERCENT | | | | OBSERVED VALUES OF $p$ FROM: | | | |
|---|---|---|---|---|---|---|---|---|
| | $a$ | $b$ | $c$ | $d$ | EMERSON'S $p$ | YULE'S $Q$ | FISHER'S OR HALDANE'S $p$ | $r$ |
| Perfect population | 56.25 | 18.75 | 18.75 | 6.25 | 50.00 | 50.00 | 50.00 | 50.00 |
| 25 percent $a$ gametes not effective | 61.22 | 20.41 | 13.78 | 4.59 | 56.24 | 50.00 | 50.00 | 50.00 |
| 25 percent $a$ and $b$ gametes not effective | 66.64 | 14.99 | 14.99 | 3.37 | 63.26 | 50.00 | 52.58 | 50.00 |
| 25 percent $a$ and $B$ gametes not effective | 54.98 | 26.66 | 12.37 | 6.00 | 46.85 | 50.00 | 47.97 | 50.00 |
| 25 percent $a$ zygotes die | 60.00 | 20.00 | 15.00 | 5.00 | 54.77 | 50.00 | 50.00 | 50.00 |
| 25 percent $a$ and $b$ zygotes die | 64.00 | 16.00 | 16.00 | 4.00 | 60.00 | 50.00 | 51.33 | 50.00 |
| 25 percent $a$ and $B$ zygotes die | 55.38 | 24.62 | 13.85 | 6.15 | 48.04 | 50.00 | 48.83 | 50.00 |
| 25 percent $a$ zygotes called $A$ | 60.94 | 20.31 | 14.06 | 4.69 | 55.90 | 50.00 | 50.00 | 50.00 |
| Double recessive class not recovered | 60.00 | 20.00 | 20.00 | 0.00 | 44.72 | 0.00 | 0.00 | 25.00 |

TABLE 4

*Comparison of methods of measuring linkage values in $F_2$—25 percent actual gametic linkage.*

| | ZYGOTIC CLASSES—IN PERCENT | | | | OBSERVED VALUES OF $p$ FROM: | | | |
|---|---|---|---|---|---|---|---|---|
| | *a* | *b* | *c* | *d* | EMERSON'S $p$ | YULE'S $Q$ | FISHER'S OR HALDANE'S $p$ | $r$ |
| **Coupling phase** | | | | | | | | |
| Perfect population | 64.06 | 10.94 | 10.94 | 14.06 | 75.00 | 75.00 | 75.00 | 75.00 |
| 25 percent *a* gametes not effective | 70.41 | 11.22 | 8.04 | 10.33 | 78.41 | 74.32 | 75.82 | 74.17 |
| 25 percent *a* and *b* gametes not effective | 76.40 | 8.08 | 8.08 | 7.44 | 82.26 | 73.59 | 78.11 | 73.33 |
| 25 percent *a* and *B* gametes not effective | 63.51 | 14.96 | 7.75 | 13.77 | 73.88 | 74.98 | 73.95 | 74.56 |
| 25 percent *a* zygotes die | 68.33 | 11.67 | 8.75 | 11.25 | 76.92 | 75.00 | 75.00 | 73.98 |
| 25 percent *a* and *b* zygotes die | 72.49 | 9.28 | 9.28 | 8.95 | 79.29 | 75.00 | 75.85 | 73.01 |
| 25 percent *a* and *B* zygotes die | 63.48 | 14.45 | 8.13 | 13.94 | 74.05 | 75.00 | 74.18 | 74.68 |
| 25 percent *a* zygotes called *A* | 66.80 | 14.45 | 8.20 | 10.55 | 73.95 | 77.42 | 71.90 | 71.41 |
| One crossover class not recovered | 71.93 | 12.28 | 0.00 | 15.78 | 86.84 | 100.00 | 86.12 | 87.74 |
| **Repulsion phase** | | | | | | | | |
| Perfect population | 51.56 | 23.44 | 23.44 | 1.56 | 25.00 | 25.00 | 25.00 | 25.00 |
| 25 percent *a* gametes not effective | 54.06 | 27.55 | 17.22 | 1.15 | 32.34 | 23.77 | 23.35 | 27.10 |
| 25 percent *a* and *b* gametes not effective | 57.82 | 20.66 | 20.66 | 0.86 | 41.67 | 22.63 | 23.48 | 28.74 |
| 25 percent *a* and *B* gametes not effective | 49.22 | 35.26 | 14.05 | 1.47 | 11.74 | 24.96 | 22.87 | 26.11 |
| 25 percent *a* zygotes die | 55.00 | 25.00 | 18.75 | 1.25 | 35.36 | 25.00 | 25.00 | 28.20 |
| 25 percent *a* and *b* zygotes die | 58.86 | 20.07 | 20.07 | 1.00 | 44.42 | 25.00 | 26.18 | 30.83 |
| 25 percent *a* and *B* zygotes die | 50.57 | 30.65 | 17.24 | 1.53 | 20.53 | 25.00 | 24.14 | 25.41 |
| 25 percent *a* zygotes called *A* | 57.42 | 23.83 | 17.58 | 1.17 | 41.46 | 26.00 | 26.40 | 30.65 |
| Double recessive not recovered | 52.38 | 23.81 | 23.81 | 0.00 | 21.82 | 0.00 | 0.00 | 12.50 |

### *Yule's coefficient of association Q*

The coefficient of association $Q$ has been a source of much controversy as a measure of actual correlation (YULE 1912 and PEARSON and HERON 1913). The disadvantages of this method, as a general method, are well appreciated and the reader is referred to the discussion given by PEARSON

and HERON. However, the defects in the method as a measure of correlation really become an asset in estimating crossing over percentages in certain instances. Its use in connection with the coefficient of correlation may, therefore, be highly recommended, but certainly indiscriminate usage (as feared by PEARSON and HERON) should by all means be avoided.

The formula under discussion is

$$Q=\frac{ad-bc}{ad+bc} \tag{7}$$

The use of this formula is limited to discrete units the same as the coefficient of correlation. But granting this assumption it has some very peculiar properties which must be known before it can be properly employed.

First it should be pointed out (see illustrations in tables 2, 3 and 4) that $Q$ represents perfect negative correlation when $d=0$ and perfect positive correlation when either $b=0$ or when $c=0$. It is very obvious that this can not be true.

PEARSON and HERON have shown that a fourfold distribution may be manipulated so that $r$ will remain constant but $Q$ will rise at will and with $Q$ constant $r$ can fall to zero.

These properties clearly show the limitations of $Q$ but certain advantages arise from the following property.

The value of $Q$ is not changed by multiplying $(a+b)$, $(c+d)$, $(a+c)$, or $(c+d)$ by any factor because the fourfold table

| | | |
|---|---|---|
| $ka$ | $b$ | $ka+b$ |
| $kc$ | $d$ | $kc+d$ |
| $k(a+c)$ | $(b+d)$ | $n$ |

gives the same value for $Q$ for all values of $k$. This property is certainly a very decided advantage in the case of differential death rate of zygotes due to one of the factors under consideration acting as a lethal or semi-lethal factor.

For specific ratios there is a definite relation between $r$ and $Q$. YULE (1900) gives the following general relationship:

$$k=\frac{1-Q}{1+Q} \qquad r=\frac{\cos\sqrt{k}}{1+\sqrt{k}}\pi$$

For the Mendelian ratios (3:1 and 3:1)

$$Q=\frac{24r}{(3r+1)^2+8}$$

If $Q$ is to be used as a measure of correlation it is also very necessary to consider its probable error.

$$E_Q=.6745\times\frac{1-Q^2}{2}\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}} \tag{8}$$

because this may be different (PEARSON and HERON 1913, page 175) for the same values of $Q$ and $n$. It will be noted that this formula may be simpler than that for coefficient of correlation (see formula [3]).

Unfortunately $Q$ is not so well adapted to algebraic manipulation as the coefficient of correlation $r$ but a determination of $p$ from corresponding values of $Q$ can be made by a relatively small amount of labor.

In an $F_2$ distribution (3:1 and 3:1 ratios),[1]

$$p^2=\frac{2-\sqrt{4-2Q-2Q^2}}{2Q} \tag{9}$$

For an $F_2$ distribution with complete linkage in one sex

$$p=\frac{2-\sqrt{4-2Q-2Q^2}}{2Q} \tag{10}$$

and for backcrossing (1:1 and 1:1 ratios).

$$p=\frac{Q+1-\sqrt{1-Q^2}}{2Q} \tag{11}$$

Further combinations may be calculated in a similar manner from the theoretical values of $a''$, $b''$ $c''$ and $d''$, in terms of $p$. (See tables 5 to 15).

*Examples*

*Example* 1—(3:1 and 3:1 ratios). This example is taken from an experiment with maize by EAST and HAYES presented by BABCOCK and CLAUSEN (1918).

The observed data compared with a 9:3:3:1 distribution are as follows:

| *Purple, starchy* | *Purple, sweet* | *White, starchy* | *White, sweet* |
|---|---|---|---|
| 1,861 | 614 | 548 | 217 |
| 1,822.5 | 607.5 | 607.5 | 202.5 |

[1] When $Q=0$ it is necessary to differentiate both numerator and denominator to get the proper value for $p$.

A comparison with theoretical ratios without correlation was considered which led to $\chi^2=7.75$ and the odds against a fit as bad as this were found to be about 18:1 from ELDERTON'S tables. From a consideration of $\chi^2$ as a measure of correlation a value of $\chi^2=7.75$ would probably be very significant but by making the comparison with theoretical ratios one cannot easily see whether the deviation from a perfect fit is due mostly to aberrations in ratios or to linkage. The preferable calculation from formula [2] making $\chi^2$ a measure of correlation gives: $\chi^2=3.88$. It will be noted that this is considerably less than the value derived from the previous method showing that correlation (or linkage) alone was not the only cause of the relatively poor fit.

The coefficient of correlation from formula [1] is: $r=0.0346$. The standard deviation of $r$ considering random distribution is $\frac{1}{\sqrt{n}}$ or 0.0176. Dividing $r$ by ${}_o\sigma_r$ is the same as taking the square root of $\chi^2$. This gives $\chi=1.97$. This value may be slightly suspicious but can hardly be considered significant, but if $\chi^2$ (due to correlation) had been 7.75, then $\frac{r}{{}_o\sigma_r}$ would have been 2.78[1], a much more significant figure.

The value of $p$ (proportion of $AB$ or $ab$ gametes) may be calculated in four different ways for this particular problem without marked differences. These are as follows:

| *Method* | *Value of p in percent* |
|---|---|
| $r$ | 52.53 |
| $Q$ | 52.56 |
| FISHER'S $p$ | 52.52 |
| EMERSON'S $p$ | 53.17 |

In this example the deficiency is largely in the white, starchy class. This causes the ratios to deviate considerably from the theoretical but not in such a way as to indicate differential zygotic death rates due to either of the factors under consideration. Differential mortality due to the particular combination of factors would be a more logical expectation from the data available.

If the correlation is not significant and if larger $F_2$ populations were grown the crossing over percentage would be expected to approach 50 percent more closely. However, if increased numbers still indicated a value for $p$ of 0.525 it would finally become significant. Then the crossing over

[1] If the frequency of $r$ in terms of ${}_o\sigma_r$ were Gaussian a deviation of 2.78 times ${}_o\sigma_r$ would represent odds of 183:1. See text for a discussion of this subject. See also PEARSON'S table XV(C) (1914).

percentage would be $(1-p)\times 100$ or 47.5 percent. A comparison with theoretical ratios may be seen in tables 7 and 8.

*Example* 2—(15:1 and 3:1 ratios.)

This example is taken from data published by the writer (OWEN 1927, progeny no. 13). Very similar observations were also made by WOODWORTH (1923). Duplicate factors, $D_1$ and $D_2$, are assumed to be equally capable of producing yellow cotyledon color. The factor $D_1$ is apparently linked with $G$, the factor for green seed-coat color. The recessive characters are green-cotyledon color and yellow seed-coat color respectively. The observed $F_2$ data compared with a theoretical random distribution are as follows:

| | YELLOW COTYLEDONS | | GREEN COTYLEDONS | |
|---|---|---|---|---|
| | Green seed-coat | Yellow seed-coat | Green seed-coat | Yellow seed-coat |
| Observed | 236 | 85 | 16 | 0 |
| *Calculated* | *236.9* | *79.0* | *15.8* | *5.3* |

Calculating $\chi^2$ in the usual way by comparing the observed distribution with a theoretical distribution with 15:1 and 3:1 ratios and 50 percent crossing over gives $\chi^2=5.72$. If the data are corrected to give an exact 15:1 ratio for cotyledon color which seemed logical because of the necessity of growing $F_2$ seed to determine the color of the $F_2$ seed coats, another calculation gives $\chi^2=5.26$. It makes little difference which value is considered, that is, $\chi^2=5.27$ or 5.26. From ELDERTON'S tables the odds against a fit as bad as the observed due to chance alone are approximately 8:1. Is this significant?

In contrast with the conclusions that might be drawn from the above paragraph consider the value of $\chi^2$ from formula [2]. This is 7.6 and the odds from ELDERTON'S tables are nearly 20:1. Is this significant? Surely much more suspicion is likely to arise than from the 8:1 odds but one may be somewhat puzzled until he realizes that this value of $\chi^2$ is a measure of correlation. It was previously shown that the probability of securing the double recessive class in 337 observations threw more light on the subject. The probability of securing 16 $F_2$ seeds with green cotyledons without a single $F_2$ plant (of the 16) having yellow seed coats leads to a very definite conclusion. The odds against random distribution are $1:(3/4)^{16}$ or approximately 100:1 and additional information gained from subsequent $F_2$ populations continued to indicate a significant correlation.

If the formulae given in table 1 are used to estimate the crossing over percentage the direct calculation gives 32.00 percent while the calculation from $r$ gives 18.04 percent. Previously the writer found by inspection (see table 10) that approximately 13 percent crossing over gave a very good comparison with the observed distribution. This illustrates the usefulness of the coefficient of correlation because the direct calculation (which gave 32 percent) is very inaccurate especially in the repulsion phase. In this particular problem the coefficient of association fails entirely to express a logical crossover value. From formula [7] $Q=-1.00$ which indicates complete repulsion and would imply that $D_1$ and $g$ were completely linked.

## SUMMARY

A method has been suggested for calculating linkage intensities by means of a product moment coefficient of correlation $r$. This is recommended as a general method in preference to the other methods with which it has been compared.

YULE'S coefficient of association $Q$ is a valuable supplement to the coefficient of correlation, and when there is knowledge of differential mortality of zygotes it gives a better approximation to the true crossing over percentage.

FISHER'S method has also been found very convenient but its use is limited to distributions where observed ratios closely approach the theoretical ratios.

Tables similar to those given by COLLINS (1924) have been used to illustrate the various methods.

Much value is attached to the coefficient of correlation because it may be readily applied to complex relationships as well as to simple cases of backcrossing. Formulae for the most general cases are given and tables for theoretical distributions are included in the appendix.

The convenient relationship between $\chi^2$ and $r$ has been illustrated. When $\chi^2$ is a measure of correlation, $\chi^2=nr^2$ and $\sqrt{n}\,r^2=\chi=\frac{r}{{}_o\sigma_r}$.

Attention has been called to the fact that $r$ and $Q$, for accurate estimates, apply only to distributions made up of discrete units where the characters are clear-cut. Otherwise an error may be introduced which is not easily measured.

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## APPENDIX

### TABLE 5

*Crossing over percentages and values of r and Q for an $F_1$ backcrossed with the double recessive—1:1 and 1:1 ratios.*

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES—IN PERCENT | | | | $Q$ | $r$ |
|---|---|---|---|---|---|---|
| | $a''$ | $b''$ | $c''$ | $d''$ | | |
| | $\frac{p}{2}$ | $\frac{1-p}{2}$ | $\frac{1-p}{2}$ | $\frac{p}{2}$ | $\frac{2p-1}{2p^2-2p+1}$ | $\frac{2p-1}{1}$ |
| **Coupling phase** | | | | | | |
| 0 | 50.00 | 0.00 | 0.00 | 50.00 | 1.0000 | 1.00 |
| 5 | 47.50 | 2.50 | 2.50 | 47.50 | 0.9945 | 0.90 |
| 10 | 45.00 | 5.00 | 5.00 | 45.00 | 0.9757 | 0.80 |
| 15 | 42.50 | 7.50 | 7.50 | 42.50 | 0.9396 | 0.70 |
| 20 | 40.00 | 10.00 | 10.00 | 40.00 | 0.8824 | 0.60 |
| 25 | 37.50 | 12.50 | 12.50 | 37.50 | 0.8000 | 0.50 |
| 30 | 35.00 | 15.00 | 15.00 | 35.00 | 0.6897 | 0.40 |
| 35 | 32.50 | 17.50 | 17.50 | 32.50 | 0.5505 | 0.30 |
| 40 | 30.00 | 20.00 | 20.00 | 30.00 | 0.3846 | 0.20 |
| 45 | 27.50 | 22.50 | 22.50 | 27.50 | 0.1980 | 0.10 |
| 50 | 25.00 | 25.00 | 25.00 | 25.00 | 0.0000 | 0.00 |
| **Repulsion phase** | | | | | | |
| 0 | 0.00 | 50.00 | 50.00 | 0.00 | −1.0000 | −1.00 |
| 5 | 2.50 | 47.50 | 47.50 | 2.50 | −0.9945 | −0.90 |
| 10 | 5.00 | 45.00 | 45.00 | 5.00 | −0.9757 | −0.80 |
| 15 | 7.50 | 42.50 | 42.50 | 7.50 | −0.9396 | −0.70 |
| 20 | 10.00 | 40.00 | 40.00 | 10.00 | −0.8824 | −0.60 |
| 25 | 12.50 | 37.50 | 37.50 | 12.50 | −0.8000 | −0.50 |
| 30 | 15.00 | 35.00 | 35.00 | 15.00 | −0.6897 | −0.40 |
| 35 | 17.50 | 32.50 | 32.50 | 17.50 | −0.5505 | −0.30 |
| 40 | 20.00 | 30.00 | 30.00 | 20.00 | −0.3846 | −0.20 |
| 45 | 22.50 | 27.50 | 27.50 | 22.50 | −0.1980 | −0.10 |
| 50 | 25.00 | 25.00 | 25.00 | 25.00 | 0.0000 | 0.00 |

TABLE 6

*Crossing over percentages when there is complete linkage in one sex—3:1 and 3:1 ratios*

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES—PERCENT | | | | $Q$ | $r$ |
|---|---|---|---|---|---|---|
| | $a''$ | $b''$ | $c''$ | $d''$ | | |
| | $\frac{2+p}{4}$ | $\frac{1-p}{4}$ | $\frac{1-p}{4}$ | $\frac{p}{4}$ | $\frac{4p-1}{2p^2+1}$ | $\frac{4p-1}{3}$ |
| | | | Coupling phase | | | |
| 0 | 75.00 | 0.00 | 0.00 | 25.00 | 1.0000 | 1.0000 |
| 5 | 73.75 | 1.25 | 1.25 | 23.75 | 0.9982 | 0.9333 |
| 10 | 72.50 | 2.50 | 2.50 | 22.50 | 0.9923 | 0.8667 |
| 15 | 71.25 | 3.75 | 3.75 | 21.25 | 0.9816 | 0.8000 |
| 20 | 70.00 | 5.00 | 5.00 | 20.00 | 0.9649 | 0.7333 |
| 25 | 68.75 | 6.25 | 6.25 | 18.75 | 0.9412 | 0.6667 |
| 30 | 67.50 | 7.50 | 7.50 | 17.50 | 0.9091 | 0.6000 |
| 35 | 66.25 | 8.75 | 8.75 | 16.25 | 0.8672 | 0.5333 |
| 40 | 65.00 | 10.00 | 10.00 | 15.00 | 0.8140 | 0.4667 |
| 45 | 63.75 | 11.25 | 11.25 | 13.75 | 0.7476 | 0.4000 |
| 50 | 62.50 | 12.50 | 12.50 | 12.50 | 0.6667 | 0.3333 |
| | | | Repulsion phase | | | |
| 0 | 50.00 | 25.00 | 25.00 | 0.00 | −1.0000 | −0.3333 |
| 5 | 51.25 | 23.75 | 23.75 | 1.25 | −0.7960 | −0.2667 |
| 10 | 52.50 | 22.50 | 22.50 | 2.50 | −0.5882 | −0.2000 |
| 15 | 53.75 | 21.25 | 21.25 | 3.75 | −0.3828 | −0.1333 |
| 20 | 55.00 | 20.00 | 20.00 | 5.00 | −0.1852 | −0.0667 |
| 25 | 56.25 | 18.75 | 18.75 | 6.25 | 0.0000 | 0.0000 |
| 30 | 57.50 | 17.50 | 17.50 | 7.50 | 0.1695 | 0.0667 |
| 35 | 58.75 | 16.25 | 16.25 | 8.75 | 0.3213 | 0.1333 |
| 40 | 60.00 | 15.00 | 15.00 | 10.00 | 0.4545 | 0.2000 |
| 45 | 61.25 | 13.75 | 13.75 | 11.25 | 0.5694 | 0.2667 |
| 50 | 62.50 | 12.50 | 12.50 | 12.50 | 0.6667 | 0.3333 |

TABLE 7

*Crossing over percentages and values of Q and r with corresponding theoretical $F_2$ phenotypic ratios with single factors for both characters—3:1 and 3:1 ratios—Coupling phase.*

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES—IN PERCENT | | | | Q | r |
|---|---|---|---|---|---|---|
| | a'' | b'' | c'' | d'' | COEFFICIENT OF ASSOCIATION | COEFFICIENT OF CORRELATION |
| | $\frac{2+p^2}{4}$ | $\frac{1-p^2}{4}$ | $\frac{1-p^2}{4}$ | $\frac{p^2}{4}$ | $\frac{4p^2-1}{2p^4+1}$ | $\frac{4p^2-1}{3}$ |
| 0 | 75.00 | 0.00 | 0.00 | 25.00 | 1.0000 | 1.0000 |
| 1 | 74.50 | 0.50 | 0.50 | 24.50 | 0.9997 | 0.9735 |
| 2 | 74.01 | 0.99 | 0.99 | 24.01 | 0.9989 | 0.9472 |
| 3 | 73.52 | 1.48 | 1.48 | 23.52 | 0.9975 | 0.9212 |
| 4 | 73.04 | 1.96 | 1.96 | 23.04 | 0.9954 | 0.8955 |
| 5 | 72.56 | 2.44 | 2.44 | 22.56 | 0.9928 | 0.8700 |
| 6 | 72.09 | 2.91 | 2.91 | 22.09 | 0.9894 | 0.8448 |
| 7 | 71.62 | 3.38 | 3.38 | 21.62 | 0.9854 | 0.8199 |
| 8 | 71.16 | 3.84 | 3.84 | 21.16 | 0.9806 | 0.7952 |
| 9 | 70.70 | 4.30 | 4.30 | 20.70 | 0.9751 | 0.7708 |
| 10 | 70.25 | 4.75 | 4.75 | 20.25 | 0.9688 | 0.7467 |
| 11 | 69.80 | 5.20 | 5.20 | 19.80 | 0.9617 | 0.7228 |
| 12 | 69.36 | 5.64 | 5.64 | 19.36 | 0.9537 | 0.6992 |
| 13 | 68.92 | 6.08 | 6.08 | 18.92 | 0.9449 | 0.6759 |
| 14 | 68.49 | 6.51 | 6.51 | 18.49 | 0.9352 | 0.6528 |
| 15 | 68.06 | 6.94 | 6.94 | 18.06 | 0.9247 | 0.6300 |
| 16 | 67.64 | 7.36 | 7.36 | 17.64 | 0.9131 | 0.6075 |
| 17 | 67.22 | 7.78 | 7.78 | 17.22 | 0.9007 | 0.5852 |
| 18 | 66.81 | 8.19 | 8.19 | 16.81 | 0.8873 | 0.5632 |
| 19 | 66.40 | 8.60 | 8.60 | 16.40 | 0.8729 | 0.5415 |
| 20 | 66.00 | 9.00 | 9.00 | 16.00 | 0.8575 | 0.5200 |
| 21 | 65.60 | 9.40 | 9.40 | 15.60 | 0.8411 | 0.4988 |
| 22 | 65.21 | 9.79 | 9.79 | 15.21 | 0.8238 | 0.4779 |
| 23 | 64.82 | 10.18 | 10.18 | 14.82 | 0.8054 | 0.4572 |
| 24 | 64.44 | 10.56 | 10.56 | 14.44 | 0.7860 | 0.4368 |
| 25 | 64.06 | 10.94 | 10.94 | 14.06 | 0.7656 | 0.4167 |
| 26 | 63.69 | 11.31 | 11.31 | 13.69 | 0.7441 | 0.3968 |
| 27 | 63.32 | 11.68 | 11.68 | 13.32 | 0.7217 | 0.3772 |
| 28 | 62.96 | 12.04 | 12.04 | 12.96 | 0.6983 | 0.3579 |
| 29 | 62.60 | 12.40 | 12.40 | 12.60 | 0.6739 | 0.3388 |
| 30 | 62.25 | 12.75 | 12.75 | 12.25 | 0.6486 | 0.3200 |
| 31 | 61.90 | 13.10 | 13.10 | 11.90 | 0.6223 | 0.3015 |
| 32 | 61.56 | 13.44 | 13.44 | 11.56 | 0.5951 | 0.2832 |
| 33 | 61.22 | 13.78 | 13.78 | 11.22 | 0.5671 | 0.2652 |
| 34 | 60.89 | 14.11 | 14.11 | 10.89 | 0.5382 | 0.2475 |
| 35 | 60.56 | 14.44 | 14.44 | 10.56 | 0.5085 | 0.2300 |
| 36 | 60.24 | 14.76 | 14.76 | 10.24 | 0.4780 | 0.2128 |
| 37 | 59.92 | 15.08 | 15.08 | 9.92 | 0.4468 | 0.1959 |
| 38 | 59.61 | 15.39 | 15.39 | 9.61 | 0.4150 | 0.1792 |
| 39 | 59.30 | 15.70 | 15.70 | 9.30 | 0.3825 | 0.1628 |

TABLE 7—continued

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES—IN PERCENT | | | | $Q$ | $r$ |
|---|---|---|---|---|---|---|
| | $a''$ | $b''$ | $c''$ | $d''$ | COEFFICIENT OF ASSOCIATION | COEFFICIENT OF CORRELATION |
| | $\frac{2+p^2}{4}$ | $\frac{1-p^2}{4}$ | $\frac{1-p^2}{4}$ | $\frac{p^2}{4}$ | $\frac{4p^2-1}{2p^4+1}$ | $\frac{4p^2-1}{3}$ |
| 40 | 59.00 | 16.00 | 16.00 | 9.00 | 0.3494 | 0.1467 |
| 41 | 58.70 | 16.30 | 16.30 | 8.70 | 0.3159 | 0.1308 |
| 42 | 58.41 | 16.59 | 16.59 | 8.41 | 0.2818 | 0.1152 |
| 43 | 58.12 | 16.88 | 16.88 | 8.12 | 0.2474 | 0.0999 |
| 44 | 57.84 | 17.16 | 17.16 | 7.84 | 0.2126 | 0.0848 |
| 45 | 57.56 | 17.44 | 17.44 | 7.56 | 0.1775 | 0.0700 |
| 46 | 57.29 | 17.71 | 17.71 | 7.29 | 0.1422 | 0.0555 |
| 47 | 57.02 | 17.98 | 17.98 | 7.02 | 0.1068 | 0.0412 |
| 48 | 56.76 | 18.24 | 18.24 | 6.76 | 0.0712 | 0.0272 |
| 49 | 56.50 | 18.50 | 18.50 | 6.50 | 0.0356 | 0.0135 |
| 50 | 56.25 | 18.75 | 18.75 | 6.25 | 0.0000 | 0.0000 |

TABLE 8

*Crossing over percentages and values of Q and r with corresponding theoretical $F_2$ phenotypic ratios with single factors for both characters—3:1 and 3:1 ratios—Repulsion phase.*

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES—IN PERCENT | | | | $Q$ | $r$ |
|---|---|---|---|---|---|---|
| | $a''$ | $b''$ | $c''$ | $d''$ | COEFFICIENT OF ASSOCIATION | COEFFICIENT OF CORRELATION |
| | $\frac{2+p^2}{4}$ | $\frac{1-p^2}{4}$ | $\frac{1-p^2}{4}$ | $\frac{p^2}{4}$ | $\frac{4p^2-1}{2p^4+1}$ | $\frac{4p^2-1}{3}$ |
| 0 | 50.00 | 25.00 | 25.00 | 0.00 | −1.0000 | −0.3333 |
| 1 | 50.00 | 25.00 | 25.00 | 0.00 | −0.9996 | −0.3332 |
| 2 | 50.01 | 24.99 | 24.99 | 0.01 | −0.9984 | −0.3328 |
| 3 | 50.02 | 24.98 | 24.98 | 0.02 | −0.9964 | −0.3321 |
| 4 | 50.04 | 24.96 | 24.96 | 0.04 | −0.9936 | −0.3312 |
| 5 | 50.06 | 24.94 | 24.94 | 0.06 | −0.9900 | −0.3300 |
| 6 | 50.09 | 24.91 | 24.91 | 0.09 | −0.9856 | −0.3285 |
| 7 | 50.12 | 24.88 | 24.88 | 0.12 | −0.9804 | −0.3268 |
| 8 | 50.16 | 24.84 | 24.84 | 0.16 | −0.9743 | −0.3248 |
| 9 | 50.20 | 24.80 | 24.80 | 0.20 | −0.9675 | −0.3225 |
| 10 | 50.25 | 24.75 | 24.75 | 0.25 | −0.9598 | −0.3200 |
| 11 | 50.30 | 24.70 | 24.70 | 0.30 | −0.9513 | −0.3172 |
| 12 | 50.36 | 24.64 | 24.64 | 0.36 | −0.9420 | −0.3141 |
| 13 | 50.42 | 24.58 | 24.58 | 0.42 | −0.9319 | −0.3108 |
| 14 | 50.49 | 24.51 | 24.51 | 0.49 | −0.9209 | −0.3072 |
| 15 | 50.56 | 24.44 | 24.44 | 0.56 | −0.9091 | −0.3033 |
| 16 | 50.64 | 24.36 | 24.36 | 0.64 | −0.8964 | −0.2992 |

TABLE 8—continued

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES—IN PERCENT | | | | $Q$ | $r$ |
|---|---|---|---|---|---|---|
| | $a''$ | $b''$ | $c''$ | $d''$ | COEFFICIENT OF ASSOCIATION | COEFFICIENT OF CORRELATION |
| | $\frac{2+p^2}{4}$ | $\frac{1-p^2}{4}$ | $\frac{1-p^2}{4}$ | $\frac{p^2}{4}$ | $\frac{4p^2-1}{2p^4+1}$ | $\frac{4p^2-1}{3}$ |
| 17 | 50.72 | 24.28 | 24.28 | 0.72 | −0.8829 | −0.2948 |
| 18 | 50.81 | 24.19 | 24.19 | 0.81 | −0.8686 | −0.2901 |
| 19 | 50.90 | 24.10 | 24.10 | 0.90 | −0.8534 | −0.2852 |
| 20 | 51.00 | 24.00 | 24.00 | 1.00 | −0.8373 | −0.2800 |
| 21 | 51.10 | 23.90 | 23.90 | 1.10 | −0.8204 | −0.2745 |
| 22 | 51.21 | 23.79 | 23.79 | 1.21 | −0.8026 | −0.2688 |
| 23 | 51.32 | 23.68 | 23.68 | 1.32 | −0.7840 | −0.2628 |
| 24 | 51.44 | 23.56 | 23.56 | 1.44 | −0.7645 | −0.2565 |
| 25 | 51.56 | 23.44 | 23.44 | 1.56 | −0.7442 | −0.2500 |
| 26 | 51.69 | 23.31 | 23.31 | 1.69 | −0.7230 | −0.2432 |
| 27 | 51.82 | 23.18 | 23.18 | 1.82 | −0.7009 | −0.2361 |
| 28 | 51.96 | 23.04 | 23.04 | 1.96 | −0.6781 | −0.2288 |
| 29 | 52.10 | 22.90 | 22.90 | 2.10 | −0.6543 | −0.2212 |
| 30 | 52.25 | 22.75 | 22.75 | 2.25 | −0.6298 | −0.2133 |
| 31 | 52.40 | 22.60 | 22.60 | 2.40 | −0.6044 | −0.2052 |
| 32 | 52.56 | 22.44 | 22.44 | 2.56 | −0.5783 | −0.1968 |
| 33 | 52.72 | 22.28 | 22.28 | 2.72 | −0.5513 | −0.1881 |
| 34 | 52.89 | 22.11 | 22.11 | 2.89 | −0.5236 | −0.1792 |
| 35 | 53.06 | 21.94 | 21.94 | 3.06 | −0.4951 | −0.1700 |
| 36 | 53.24 | 21.76 | 21.76 | 3.24 | −0.4659 | −0.1605 |
| 37 | 53.42 | 21.58 | 21.58 | 3.42 | −0.4361 | −0.1508 |
| 38 | 53.61 | 21.39 | 21.39 | 3.61 | −0.4055 | −0.1408 |
| 39 | 53.80 | 21.20 | 21.20 | 3.80 | −0.3743 | −0.1305 |
| 40 | 54.00 | 21.00 | 21.00 | 4.00 | −0.3425 | −0.1200 |
| 41 | 54.20 | 20.80 | 20.80 | 4.20 | −0.3101 | −0.1092 |
| 42 | 54.41 | 20.59 | 20.59 | 4.41 | −0.2772 | −0.0981 |
| 43 | 54.62 | 20.38 | 20.38 | 4.62 | −0.2437 | −0.0868 |
| 44 | 54.84 | 20.16 | 20.16 | 4.84 | −0.2099 | −0.0752 |
| 45 | 55.06 | 19.94 | 19.94 | 5.06 | −0.1756 | −0.0633 |
| 46 | 55.29 | 19.71 | 19.71 | 5.29 | −0.1410 | −0.0512 |
| 47 | 55.52 | 19.48 | 19.48 | 5.52 | −0.1061 | −0.0388 |
| 48 | 55.76 | 19.24 | 19.24 | 5.76 | −0.0709 | −0.0261 |
| 49 | 56.00 | 19.00 | 19.00 | 6.00 | −0.0355 | −0.0132 |
| 50 | 56.25 | 18.75 | 18.75 | 6.25 | 0.0000 | 0.0000 |

TABLE 9

*Crossing over percentages and values of r when one character depends upon complementary factors—9:7 and 3:1 ratios.*

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES— IN PERCENT | | | | r |
|---|---|---|---|---|---|
| | $a''$ | $b''$ | $c''$ | $d''$ | |
| | $\frac{6+3p^2}{16}$ | $\frac{3-3p^2}{16}$ | $\frac{6-3p^2}{16}$ | $\frac{1+3p^2}{16}$ | $\frac{4p^2-1}{\sqrt{21}}$ |
| Coupling phase | | | | | |
| 0 | 56.25 | 0.00 | 18.75 | 25.00 | 0.6547 |
| 5 | 54.42 | 1.83 | 20.58 | 23.17 | 0.5695 |
| 10 | 52.69 | 3.56 | 22.31 | 21.44 | 0.4888 |
| 15 | 51.05 | 5.20 | 23.95 | 19.80 | 0.4124 |
| 20 | 49.50 | 6.75 | 25.50 | 18.25 | 0.3404 |
| 25 | 48.05 | 8.20 | 26.95 | 16.80 | 0.2728 |
| 30 | 46.69 | 9.56 | 28.31 | 15.44 | 0.2095 |
| 35 | 45.42 | 10.83 | 29.58 | 14.17 | 0.1506 |
| 40 | 44.25 | 12.00 | 30.75 | 13.00 | 0.0960 |
| 45 | 43.17 | 13.08 | 31.83 | 11.92 | 0.0458 |
| 50 | 42.19 | 14.06 | 32.81 | 10.94 | 0.0000 |
| Repulsion phase | | | | | |
| 0 | 37.50 | 18.75 | 37.50 | 6.25 | −0.2182 |
| 5 | 37.55 | 18.70 | 37.45 | 6.30 | −0.2160 |
| 10 | 37.69 | 18.56 | 37.31 | 6.44 | −0.2095 |
| 15 | 37.92 | 18.33 | 37.08 | 6.67 | −0.1986 |
| 20 | 38.25 | 18.63 | 36.75 | 7.00 | −0.1833 |
| 25 | 38.67 | 17.58 | 36.33 | 7.42 | −0.1637 |
| 30 | 39.19 | 17.06 | 35.81 | 7.94 | −0.1397 |
| 35 | 39.80 | 16.45 | 35.20 | 8.55 | −0.1113 |
| 40 | 40.50 | 15.75 | 34.50 | 9.25 | −0.0786 |
| 45 | 41.30 | 14.95 | 33.70 | 10.05 | −0.0415 |
| 50 | 42.19 | 14.06 | 32.81 | 10.94 | 0.0000 |

TABLE 10

*Crossing over percentages and values of r when one character is determined by duplicate factors—15:1 and 3:1 ratios.*

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES—IN PERCENT | | | | r |
|---|---|---|---|---|---|
| | $a''$ | $b''$ | $c''$ | $d''$ | |
| | $\frac{11+p^2}{16}$ | $\frac{4-p^2}{16}$ | $\frac{1-p^2}{16}$ | $\frac{p^2}{16}$ | $\frac{4p^2-1}{3\sqrt{5}}$ |
| Coupling phase | | | | | |
| 0 | 75.00 | 18.75 | 0.00 | 6.25 | 0.4472 |
| 5 | 74.39 | 19.36 | 0.61 | 5.64 | 0.3891 |
| 10 | 73.81 | 19.94 | 1.19 | 5.06 | 0.3339 |
| 15 | 73.27 | 20.48 | 1.73 | 4.52 | 0.2817 |
| 20 | 72.75 | 21.00 | 2.25 | 4.00 | 0.2326 |
| 25 | 72.27 | 21.48 | 2.73 | 3.52 | 0.1863 |
| 30 | 71.81 | 21.94 | 3.19 | 3.06 | 0.1431 |
| 35 | 71.39 | 22.36 | 3.61 | 2.64 | 0.1029 |
| 40 | 71.00 | 22.75 | 4.00 | 2.25 | 0.0656 |
| 45 | 70.64 | 23.11 | 4.36 | 1.89 | 0.0313 |
| 50 | 70.31 | 23.44 | 4.69 | 1.56 | 0.0000 |
| Repulsion phase | | | | | |
| 0 | 68.75 | 25.00 | 6.25 | 0.00 | −0.1491 |
| 5 | 68.77 | 24.98 | 6.23 | 0.02 | −0.1476 |
| 10 | 68.81 | 24.94 | 6.19 | 0.06 | −0.1431 |
| 15 | 68.89 | 24.86 | 6.11 | 0.14 | −0.1357 |
| 20 | 69.00 | 24.75 | 6.00 | 0.25 | −0.1252 |
| 25 | 69.14 | 24.61 | 5.86 | 0.39 | −0.1118 |
| 30 | 69.31 | 24.44 | 5.69 | 0.56 | −0.0954 |
| 35 | 69.52 | 24.23 | 5.48 | 0.77 | −0.0760 |
| 40 | 69.75 | 24.00 | 5.25 | 1.00 | −0.0537 |
| 45 | 70.02 | 23.74 | 4.98 | 1.27 | −0.0283 |
| 50 | 70.31 | 23.44 | 4.69 | 1.56 | 0.0000 |

TABLE 11

*Crossing over percentages and values of r when one character depends upon three supplementary factors—27:37 and 3:1 ratios.*

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES—IN PERCENT | | | | $r$ |
|---|---|---|---|---|---|
| | $a''$ | $b''$ | $c''$ | $d''$ | |
| | $\frac{18+9p^2}{64}$ | $\frac{9-9p^2}{64}$ | $\frac{30-9p^2}{64}$ | $\frac{7+9p^2}{64}$ | $\frac{4p^2-1}{\sqrt{37}}$ |
| Coupling phase | | | | | |
| 0 | 42.19 | 0.00 | 32.81 | 25.00 | 0.4932 |
| 5 | 40.82 | 1.37 | 34.18 | 23.63 | 0.4291 |
| 10 | 39.52 | 2.67 | 35.48 | 22.33 | 0.3683 |
| 15 | 38.29 | 3.90 | 36.71 | 21.10 | 0.3107 |
| 20 | 37.13 | 5.06 | 37.87 | 19.94 | 0.2565 |
| 25 | 36.04 | 6.15 | 38.96 | 18.85 | 0.2055 |
| 30 | 35.02 | 7.17 | 39.98 | 17.83 | 0.1578 |
| 35 | 34.07 | 8.12 | 40.93 | 16.88 | 0.1134 |
| 40 | 33.19 | 9.00 | 41.81 | 16.00 | 0.0723 |
| 45 | 32.38 | 9.81 | 42.62 | 15.19 | 0.0345 |
| 50 | 31.64 | 10.55 | 43.36 | 14.45 | 0.0000 |
| Repulsion phase | | | | | |
| 0 | 28.13 | 14.06 | 46.87 | 10.94 | −0.1644 |
| 5 | 28.16 | 14.03 | 46.84 | 10.97 | −0.1628 |
| 10 | 28.27 | 13.92 | 46.73 | 11.08 | −0.1578 |
| 15 | 28.44 | 13.75 | 46.56 | 11.25 | −0.1496 |
| 20 | 28.69 | 13.50 | 46.31 | 11.50 | −0.1381 |
| 25 | 29.00 | 13.18 | 46.00 | 11.82 | −0.1233 |
| 30 | 29.39 | 12.80 | 45.61 | 12.20 | −0.1052 |
| 35 | 29.85 | 12.34 | 45.15 | 12.66 | −0.0838 |
| 40 | 30.37 | 11.81 | 44.63 | 13.19 | −0.0592 |
| 45 | 30.97 | 11.21 | 44.03 | 13.79 | −0.0312 |
| 50 | 31.64 | 10.55 | 43.36 | 14.45 | 0.0000 |

TABLE 12

*Crossing over percentages when one character is determined by triplicate factors—63:1 and 3:1 ratios.*

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES—IN PERCENT | | | | $r$ |
|---|---|---|---|---|---|
| | $a''$ | $b''$ | $c''$ | $d'''$ | |
| | $\frac{47+p^2}{64}$ | $\frac{16-p^2}{64}$ | $\frac{1-p^2}{64}$ | $\frac{p^2}{64}$ | $\frac{4p^2-1}{3\sqrt{21}}$ |
| | | | Coupling phase | | |
| 0 | 75.00 | 23.44 | 0.00 | 1.56 | 0.2182 |
| 5 | 74.85 | 23.59 | 0.15 | 1.41 | 0.1899 |
| 10 | 74.70 | 23.73 | 0.30 | 1.27 | 0.1629 |
| 15 | 74.57 | 23.87 | 0.43 | 1.13 | 0.1375 |
| 20 | 74.44 | 24.00 | 0.56 | 1.00 | 0.1135 |
| 25 | 74.32 | 24.12 | 0.68 | 0.88 | 0.0909 |
| 30 | 74.20 | 24.23 | 0.80 | 0.77 | 0.0698 |
| 35 | 74.10 | 24.34 | 0.90 | 0.66 | 0.0502 |
| 40 | 74.00 | 24.44 | 1.00 | 0.56 | 0.0320 |
| 45 | 73.91 | 24.53 | 1.09 | 0.47 | 0.0153 |
| 50 | 73.83 | 24.61 | 1.17 | 0.39 | 0.0000 |
| | | | Repulsion phase | | |
| 0 | 73.44 | 25.00 | 1.56 | 0.00 | −0.0728 |
| 5 | 73.44 | 25.00 | 1.56 | 0.00 | −0.0720 |
| 10 | 73.45 | 24.98 | 1.55 | 0.02 | −0.0698 |
| 15 | 73.47 | 24.96 | 1.53 | 0.04 | −0.0662 |
| 20 | 73.50 | 24.94 | 1.50 | 0.06 | −0.0611 |
| 25 | 73.54 | 24.90 | 1.46 | 0.10 | −0.0546 |
| 30 | 73.58 | 24.86 | 1.42 | 0.14 | −0.0466 |
| 35 | 73.63 | 24.81 | 1.37 | 0.19 | −0.0371 |
| 40 | 73.69 | 24.75 | 1.31 | 0.25 | −0.0262 |
| 45 | 73.75 | 24.68 | 1.25 | 0.32 | −0.0138 |
| 50 | 73.83 | 24.61 | 1.17 | 0.39 | 0.0000 |

TABLE 13

*Crossing over percentages and values of r when both characters depend upon complementary factors—9:7 and 9:7 ratios.*

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES—IN PERCENT | | | | r |
|---|---|---|---|---|---|
| | $a''$ | $b''$ | $c''$ | $d''$ | |
| | $\frac{18+9p^2}{64}$ | $\frac{18-9p^2}{64}$ | $\frac{18-9p^2}{64}$ | $\frac{10+9p^2}{64}$ | $\frac{4p^2-1}{7}$ |
| Coupling phase | | | | | |
| 0 | 42.19 | 14.06 | 14.06 | 29.69 | 0.4286 |
| 5 | 40.82 | 15.43 | 15.43 | 28.32 | 0.3729 |
| 10 | 39.52 | 16.73 | 16.73 | 27.02 | 0.3200 |
| 15 | 38.29 | 17.96 | 17.96 | 25.79 | 0.2700 |
| 20 | 37.13 | 19.13 | 19.13 | 24.63 | 0.2229 |
| 25 | 36.04 | 20.21 | 20.21 | 23.54 | 0.1786 |
| 30 | 35.02 | 21.23 | 21.23 | 22.52 | 0.1371 |
| 35 | 34.07 | 22.18 | 22.18 | 21.57 | 0.0986 |
| 40 | 33.19 | 23.06 | 23.06 | 20.69 | 0.0629 |
| 45 | 32.38 | 23.87 | 23.87 | 19.88 | 0.0300 |
| 50 | 31.64 | 24.61 | 24.61 | 19.14 | 0.0000 |
| Repulsion phase | | | | | |
| 0 | 28.13 | 28.13 | 28.13 | 15.63 | −0.1429 |
| 5 | 28.16 | 28.09 | 28.09 | 15.66 | −0.1414 |
| 10 | 28.27 | 27.98 | 27.98 | 15.77 | −0.1371 |
| 15 | 28.44 | 27.81 | 27.81 | 15.94 | −0.1300 |
| 20 | 28.69 | 27.56 | 27.56 | 16.19 | −0.1200 |
| 25 | 29.00 | 27.25 | 27.25 | 16.50 | −0.1071 |
| 30 | 29.39 | 26.86 | 26.86 | 16.89 | −0.0914 |
| 35 | 29.85 | 26.40 | 26.40 | 17.35 | −0.0729 |
| 40 | 30.37 | 25.87 | 25.87 | 17.87 | −0.0514 |
| 45 | 30.97 | 25.28 | 25.28 | 18.47 | −0.0271 |
| 50 | 31.64 | 24.61 | 24.61 | 19.14 | 0.0000 |

TABLE 14

*Crossing over percentages and values of r when one character is determined by complementary factors and the other by duplicate factors—9:7 and 15:1 ratios.*

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES—IN PERCENT | | | | $r$ |
|---|---|---|---|---|---|
| | $a''$ | $b''$ | $c''$ | $d''$ | |
| | $\frac{33+3p^2}{64}$ | $\frac{3-3p^2}{64}$ | $\frac{27-3p^2}{64}$ | $\frac{1+3p^2}{64}$ | $\frac{4p^2-1}{\sqrt{105}}$ |
| Coupling phase | | | | | |
| 0 | 56.25 | 0.00 | 37.50 | 6.25 | 0.2928 |
| 5 | 55.79 | 0.45 | 37.95 | 5.79 | 0.2547 |
| 10 | 55.36 | 0.89 | 38.39 | 5.36 | 0.2186 |
| 15 | 54.95 | 1.30 | 38.80 | 4.95 | 0.1844 |
| 20 | 54.56 | 1.69 | 39.19 | 4.56 | 0.1523 |
| 25 | 54.20 | 2.05 | 39.55 | 4.20 | 0.1220 |
| 30 | 53.86 | 2.39 | 39.89 | 3.86 | 0.0937 |
| 35 | 53.54 | 2.71 | 40.21 | 3.54 | 0.0673 |
| 40 | 53.25 | 3.00 | 40.50 | 3.25 | 0.0429 |
| 45 | 52.98 | 3.27 | 40.77 | 2.98 | 0.0205 |
| 50 | 52.73 | 3.52 | 41.02 | 2.73 | 0.0000 |
| Repulsion phase | | | | | |
| 0 | 51.56 | 4.69 | 42.19 | 1.56 | −0.0976 |
| 5 | 51.57 | 4.68 | 42.18 | 1.57 | −0.0966 |
| 10 | 51.61 | 4.64 | 42.14 | 1.61 | −0.0937 |
| 15 | 51.67 | 4.58 | 42.08 | 1.67 | −0.0888 |
| 20 | 51.75 | 4.50 | 42.00 | 1.75 | −0.0820 |
| 25 | 51.86 | 4.39 | 41.89 | 1.86 | −0.0732 |
| 30 | 51.98 | 4.27 | 41.77 | 1.98 | −0.0625 |
| 35 | 52.14 | 4.11 | 41.61 | 2.14 | −0.0498 |
| 40 | 52.31 | 3.94 | 41.44 | 2.31 | −0.0351 |
| 45 | 52.51 | 3.74 | 41.24 | 2.51 | −0.0185 |
| 50 | 52.73 | 3.52 | 41.02 | 2.73 | 0.0000 |

TABLE 15

*Crossing over percentages and values of r when both characters are determined by duplicate factors—15:1 and 15:1 ratios.*

| PERCENT CROSSING OVER | PHENOTYPIC CLASSES—IN PERCENT | | | | r |
|---|---|---|---|---|---|
| | $a''$ | $b''$ | $c''$ | $d''$ | |
| | $\frac{56+p^2}{64}$ | $\frac{4-p^2}{64}$ | $\frac{4-p^2}{64}$ | $\frac{p^2}{64}$ | $\frac{4p^2-1}{15}$ |
| Coupling phase | | | | | |
| 0 | 89.06 | 4.69 | 4.69 | 1.56 | 0.2000 |
| 5 | 88.91 | 4.84 | 4.84 | 1.41 | 0.1740 |
| 10 | 88.77 | 4.98 | 4.98 | 1.27 | 0.1493 |
| 15 | 88.63 | 5.12 | 5.12 | 1.13 | 0.1260 |
| 20 | 88.50 | 5.25 | 5.25 | 1.00 | 0.1040 |
| 25 | 88.38 | 5.37 | 5.37 | 0.88 | 0.0833 |
| 30 | 88.27 | 5.48 | 5.48 | 0.77 | 0.0640 |
| 35 | 88.16 | 5.59 | 5.59 | 0.66 | 0.0460 |
| 40 | 88.06 | 5.69 | 5.69 | 0.56 | 0.0293 |
| 45 | 87.97 | 5.78 | 5.78 | 0.47 | 0.0140 |
| 50 | 87.89 | 5.86 | 5.86 | 0.39 | 0.0000 |
| Repulsion phase | | | | | |
| 0 | 87.50 | 6.25 | 6.25 | 0.00 | −0.0667 |
| 5 | 87.50 | 6.25 | 6.25 | 0.00 | −0.0660 |
| 10 | 87.52 | 6.23 | 6.23 | 0.02 | −0.0640 |
| 15 | 87.54 | 6.21 | 6.21 | 0.04 | −0.0607 |
| 20 | 87.56 | 6.19 | 6.19 | 0.06 | −0.0560 |
| 25 | 87.60 | 6.15 | 6.15 | 0.10 | −0.0500 |
| 30 | 87.64 | 6.11 | 6.11 | 0.14 | −0.0427 |
| 35 | 87.69 | 6.06 | 6.06 | 0.19 | −0.0340 |
| 40 | 87.75 | 6.00 | 6.00 | 0.25 | −0.0240 |
| 45 | 87.82 | 5.93 | 5.93 | 0.32 | −0.0127 |
| 50 | 87.89 | 5.86 | 5.86 | 0.39 | 0.0000 |

TABLE 16

*The influence of linkage upon Mendelian ratios.*[1]

| PERCENT CROSSING OVER | COMPLEMENTARY FACTORS | | THREE SUPPLEMENTARY FACTORS | | DUPLICATE FACTORS | | TRIPLICATE FACTORS | |
|---|---|---|---|---|---|---|---|---|
| | $\frac{2+p^2}{4}$ | $\frac{2-p^2}{4}$ | $\frac{6+3p^2}{16}$ | $\frac{10-3p^2}{16}$ | $\frac{4-p^2}{4}$ | $\frac{p^2}{4}$ | $\frac{16-p^2}{16}$ | $\frac{p^2}{16}$ |
| Coupling phase | | | | | | | | |
| 0 | 75.00 | 25.00 | 56.25 | 43.75 | 75.00 | 25.00 | 93.75 | 6.25 |
| 5 | 72.56 | 27.44 | 54.42 | 45.58 | 77.44 | 22.56 | 94.36 | 5.64 |
| 10 | 70.25 | 29.75 | 52.69 | 47.31 | 79.75 | 20.25 | 94.94 | 5.06 |
| 15 | 68.06 | 31.94 | 51.05 | 48.95 | 81.94 | 18.06 | 95.48 | 4.52 |
| 20 | 66.00 | 34.00 | 49.50 | 50.50 | 84.00 | 16.00 | 96.00 | 4.00 |
| 25 | 64.06 | 35.94 | 48.05 | 51.95 | 85.94 | 14.06 | 96.48 | 3.52 |
| 30 | 62.26 | 37.75 | 46.69 | 53.31 | 87.75 | 12.25 | 96.94 | 3.06 |
| 35 | 60.56 | 39.44 | 45.42 | 54.58 | 89.44 | 10.56 | 97.36 | 2.64 |
| 40 | 59.00 | 41.00 | 44.25 | 55.75 | 91.00 | 9.00 | 97.75 | 2.25 |
| 45 | 57.56 | 42.44 | 43.17 | 56.83 | 92.44 | 7.56 | 98.11 | 1.89 |
| 50 | 56.25 | 43.75 | 42.19 | 57.81 | 93.75 | 6.25 | 98.44 | 1.56 |
| Repulsion phase | | | | | | | | |
| 0 | 50.00 | 50.00 | 37.50 | 62.50 | 100.00 | 0.00 | 100.00 | 0.00 |
| 5 | 50.06 | 49.94 | 37.55 | 62.45 | 99.94 | 0.06 | 99.98 | 0.02 |
| 10 | 50.25 | 49.75 | 37.69 | 62.31 | 99.75 | 0.25 | 99.94 | 0.06 |
| 15 | 50.56 | 49.44 | 37.92 | 62.08 | 99.44 | 0.56 | 99.86 | 0.14 |
| 20 | 51.00 | 49.00 | 38.25 | 61.75 | 99.00 | 1.00 | 99.75 | 0.25 |
| 25 | 51.56 | 48.44 | 38.67 | 61.33 | 98.44 | 1.56 | 99.61 | 0.39 |
| 30 | 52.25 | 47.75 | 39.19 | 60.81 | 97.75 | 2.25 | 99.44 | 0.56 |
| 35 | 53.06 | 46.94 | 39.80 | 60.20 | 96.94 | 3.06 | 99.23 | 0.77 |
| 40 | 54.00 | 46.00 | 40.50 | 59.50 | 96.00 | 4.00 | 99.00 | 1.00 |
| 45 | 55.06 | 44.94 | 41.30 | 58.70 | 94.94 | 5.06 | 98.73 | 1.27 |
| 50 | 56.25 | 43.75 | 42.19 | 57.81 | 93.75 | 6.25 | 98.44 | 1.56 |

[1] When the factorial relationship is known as well as the observed ratio, the percent crossing over can be read directly. The effect of linkage of more than two factors is not considered but this would be possible when three factors are involved in the expression of one character.